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IN MEMORIAM*

POTENCIANO ARAGON Y ROSARIO: (1914–1969)

Dr. Potenciano R. Aragon, Professor of Microbiology and Dean of the Institute of Hygiene, University of the Philippines joined his creator on June 10, 1969, a victim of coronary heart attack.

He was born in Manila on May 19, 1914 and was a product of the public school system, finishing his elementary education at Singalong Elementary School in 1928 and High School at the Arellano in 1932. Then he enrolled at the College of Liberal Arts, University of the Philippines for a 2-year preparatory medicine which he finished in 1935. He then proceeded to take up medicine at the State University and graduated in 1940. Among his classmates were Drs. Arturo Reyes, Gloria Aragon, Jaime Aquino, Angelina Santos, Nelly Herrera, Jesus Nolasco, Mario Oca, Irineo Sunico, Antonio Tan and Florante Bocobo. As a college student he was well behaved, quiet and unassuming and minded his own business.

Shortly after graduation, the Institute of Hygiene of the State University offered him the position of Research Assistant. The following year (1941) he was sent to Johns Hopkins University School of Hygiene and Public Health as a fellow of the Rockefeller Foundation from where he earned the degree of Master of Public Health. After graduation in 1942, he

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joined the U.S. Army as First Lieutenant and was sent to undergo further training at U.S. Army Medical Schools such as the Medical Field Service School at Pennsylvania; Chemical Warfare School at Canal Zone, Panama. In a relatively short time he was promoted to the rank of Captain, then to Major.

After the war he returned to the Philippines to give service to his alma mater as Instructor in Hygiene in 1946. He was promoted to Assistant Professor and Acting Head of the Department of Sanitary Bacteriology and Immunology in 1947. From 1950–1960 he was promoted in rank from Assistant Professor to Associate, then to full Professor and Chairman of the Department of Medical Microbiology. In 1968 he was appointed Dean of the Institute of Hygiene, a position he ably held until his very untimely death. He is survived by his wife, Leonor Malay Aragon, who is presently the Dean of the College of Nursing, University of the Philippines.

He was a recipient of several fellowships. In 1955, he was sent to Johns Hopkins University as an exchange professor of the U.P.—Johns Hopkins Program sponsored by the Rockefeller Foundation and WHO. In 1968 he was a recipient of a 3-month travel grant sponsored by the Rockefeller Foundation to visit Schools of Public Health in the United States, Europe and Asia.

Aside from fellowships he was invited to several international meetings and/or congresses. In 1964 he was a delegate to the Fifth Congress of Tropical Medicine and Malaria held at Rio de Janairo. In 1965 he participated in the symposium on cholera held in Hawaii. He has about 24 scientific publications and the most recent just prior to his death was entitled "Serratiosis in a Nursery" published in this issue.

He was a member of the National Research Council of the Philippines, Philippine Medical Association, Philippine Society of Pathologists, Expert Panel on Health Laboratories of the World Health Organization, Philippine Public Health Association, Honor Society of Phi Kappa Phi, Philippine Board of Preventive Medicine and Public Health and many others.

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DR. POTENCIANO R. ARAGON

REPRODUCTION, LARVAL DEVELOPMENT, AND CULTIVATION OF SUGPO (PENAEUS MONODON FABRICIUS)*

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THREE PLATES AND FIVE TEXT FIGURES

INTRODUCTION

The present report deals on the reproduction and larval development of sugpo (*Penaeus monodon* Fabricius). The cultivation phase of the project, now in progress, shall be the subject of our next publication.

In the local traditional method of brackish-water fishpond management, sugpo has been considered as only secondary to bangos (*Chanos chanos* Forskål) which of course has been the primary product in such pond. Unlike the bangos fingerlings, which are purposely planted and cultivated with care, generally, sugpo fry enter the fishpond through the main gate only by chance. The average produce per hectare per year is 350 kilograms of bangos and only from 50 to 100 kilograms of sugpo.

Lately, with the introduction of improved techniques in aquaculture, it has been possible to harvest 2,000 kilograms of bangos per hectare per harvest, and in pure culture of sugpo as much as 500 kilograms may be produced. In view of the growing demand of sugpo both in the local and foreign markets, most fishpond owners in the Philippines are now starting to shift to pure sugpo culture. The price per kilogram in the local market is from ₱6.00 to ₱10.00, while in Japan (Tokyo Central Market), fresh prawns sell at from 7 to 30 U.S. dollars ($7.00–$30.00)[1] per kilogram. Japan alone imports around ₱92 millions worth of shrimps every year. The United States and France may also be considered as potential markets for our sugpo exports.

* Technical Report (July 1, 1969—June 30, 1970). MSU–NSDB Assisted Research Project No. 2.156.

The Philippines has large areas of mangrove swamps, which, in addition to more than 150,000 hectares of brackish-water fishponds, can be developed and/or redesigned for conversion into pure sugpo farms. The climate and general ecological conditions throughout the country is highly favorable, if not most ideal, for prawn culture because this crustacean prefers warm water with high salinity for spawning, larval development and normal growth. In Japan it has been observed that *Penaeus japonicus* stops feeding when water temperature drops down to 10°C or lower, thereby adversely affecting not only its normal growth but also the development of the prawn industry itself.

In view of all the above-mentioned favorable factors in the cultivation of sugpo in the Philippines, it is envisioned that prawn or sugpo farming will develop into a lucrative industry that would bring in much needed dollars and enhance our economic development. With the establishment of this new industry, continuous supply of sugpo post larvæ or juveniles will be required for stocking purposes to maintain year round harvest not only in maximum quantity but also of competitive quality for local and especially for foreign markets. Nature alone cannot be depended upon to supply all the needed stock of young sugpo for the expected accelerated sugpo-pond development, inspite of the available fishing grounds throughout the country, especially with the ever-increasing problem of water pollution due to poisonous effluents from heavy industries flowing into the same waters where our fishes, including sugpo, live to grow and spawn. Overfishing and the rampant use of dynamite are harmful practices, which adversely affect the lives of fishes in the inland waters. Hence, aquaculturists have to conduct further studies in order to aid nature not only for the purpose of establishing a new industry but also to conserve our sugpo fishery. Results from experiments prove that hatching of eggs under controlled conditions insure much greater survival rate of fry in comparison to the natural conditions.

The artificial culture of sugpo with the help of a hatchery is one of the main objectives of this research. More than 10,000 sugpo fry have been produced from around 1.5–million eggs laid by a mother sugpo in the Mindanao State University

[1] Shigeno, July, 1970.

Marine Research Laboratory. It is expected that as we improve on our hatchery and larval feeding techniques and with the acquisition of much needed additional facilities, we will be able to produce sugpo fry in more substantial quantities.

REVIEW OF LITERATURE

Early workers on prawns in the Philippines concentrated their efforts mainly on the taxonomy and the cultivation of sugpo. Blanco and Arriola (1937) were the first to attempt a systematic study of the prawns belonging to family Penaeidæ. Villaluz and Arriola (1938) made the same study on the other species of the same family known in Philippine waters.

Owing to the important economic role of sugpo in the fishpond industry, several articles about its cultivation had been written. Villadolid and Villaluz (1950) were the first to conduct observations and suggest various improvements regarding the culture of sugpo in the fishpond. Similar works were done by Mane, Villaluz, and Rabanal (1952); Villaluz (1953, 1965); Delmendo and Rabanal (1956); and Cases-Borja and Rabanal (1968) all of which tried to disseminate to fishpond owners improve methods of management necessary for the development of sugpo pond industry.

In Taiwan, Huang (1969) made mention of his observations on the capacity of sugpo fry to stay alive under a very wide range of water salinity, as a means of comparison with that of *Penaeus japonicus* Bate. Esguerra (1970) in his unpublished report to the Chairman, Development Bank of the Philippines, incorporated the "Feasibility Survey Report on Shrimp Cultivation on the Coast of the Philippines" by Shigeno, who mentioned the fact that sugpo is an entirely different animal compared with *Penaeus japonicus*. According to him, it is possible that sugpo spawn along the coast of inland waters of the Philippines. Tiews (1958) in his survey of the marine fishery resources reported the absence of gravid female sugpo in the offshore fishing grounds of Manila and San Miguel bays, so he presumed, like Shigeno, that mature sugpo migrate to and lay their eggs along the inland and coastal waters. Our findings tend to prove the truth of the above presumptions as we have collected mature specimens of Stages 4 and 5 (Fig. 1) not only along coastal waters but also inside the fishponds around Panguil and Iligan bays.

MATERIALS AND METHODS

Sugpo samples were gathered from the commercial catches of *baklad* located along Baroy, Lanao del Norte and Tangub City, Misamis Occidental, both places bordering Panguil Bay. These two places, which are approximately 5 kilometers apart across the bay are considered the major sources of sugpo in the area. In the collection of specimens, great care was taken to make representative samples by taking them at random before the catch were sorted out. From these samples, the following, among others, have been determined: carapace length—total length relationship; length frequency distribution; sex ratio; and ovary naturation.

Regular market surveys were also conducted in different markets around Panguil and Iligan bays in order to gather additional data especially on size measurements and sex ratio. To further augment the data, fishpond owners and caretakers, fishermen, vendors, middlemen, market stall holders and other dealers of sugpo were interviewed regularly.

Aside from the random sampling, live gravid females were collected from different fishing units around the bay and were taken to the MSU Marine Research Laboratory at Naawan, Misamis Oriental. The live specimens were stocked inside aquaria and experimental tanks where their feeding and spawning habits were observed. The periodic moltings of the specimens were recorded to serve as basis for the study of the rate of growth under controlled conditions. Oceanographic records like tidal ranges, water temperatures, salinity, currents, pH, plankton collections, bottom samples, stomach contents and other were also collected in order to determine the ecological requirements of the sugpo.

RESULTS AND DISCUSSIONS

Length-weight relationships. -Several workers considered total length from the tip of rostrum to the tip of the telson as the most appropriate measure of length in prawns. Recent findings [Nomura (1968)], however, tend to show that carapace length, from base of the eye-notch to the posterior mid-dorsal edge of the carapace, is more adaptable than total length. This is so because the rostrum and the tip of the telson are often cut off or easily damaged due to handling. The carapace length, therefore, is adapted in the present

research work as the standard measure of length and the basis for comparison with total length.

Carapace length-total length relationship.—The analysis of biometrical data are shown in Table 1. Data on carapace length were grouped into 3 mm intervals. Regression coefficient of carapace length on total length (Figs. 1 and 2) were based on 510 males and 482 females. In this case, a significant difference between the sexes was found. The equations are:

$$\text{Males: } Y = -10.4458 + 0.29084\ X$$
$$\text{Females: } Y = 9.5 + 0.289\ X$$

where X is the total length and Y is the carapace length both in mm.

Carapace length-total weight relationship.—Regression coefficicient of total weight on carapace length (Figs. 3 and 4) were calculated by least square of the logarithmic transformation using data on 510 males and 482 females, shown in Table 1. In this case, a significant difference was found between sexes. The equations are:

$$\text{Males: } \text{Log } W = -2.4344 + 2.592\ \text{Log } C$$
$$\text{Females: } \text{Log } W = -2.77562 + 2.7385\ \text{Log } C$$

where W is the total weight and C is the carapace length.

SIZE AND SEX COMPOSITION

Length frequency.—The frequencies for the period from December 1969 to March 1970 are shown in Fig. 5. The frequency groups of males were represented by sharp peaks owing to their small size range, while those for females were flattened. The shifting of the modal size of female in January may possibly be due to their migration to the spawning area leaving only the immature female prawns and the males. The shifting of the mode from January to March may be considered as their monthly rate of growth. The male mode seems to remain stationary which may mean to suggest that its growth becomes very slow at 36-mm carapace length, the modal size. Further investigations along this line is in progress.

There is a significant size disparity between the two sexes in the 4 months sample, the female attaining a bigger size. This size disparity, however, is common in other penaeids as

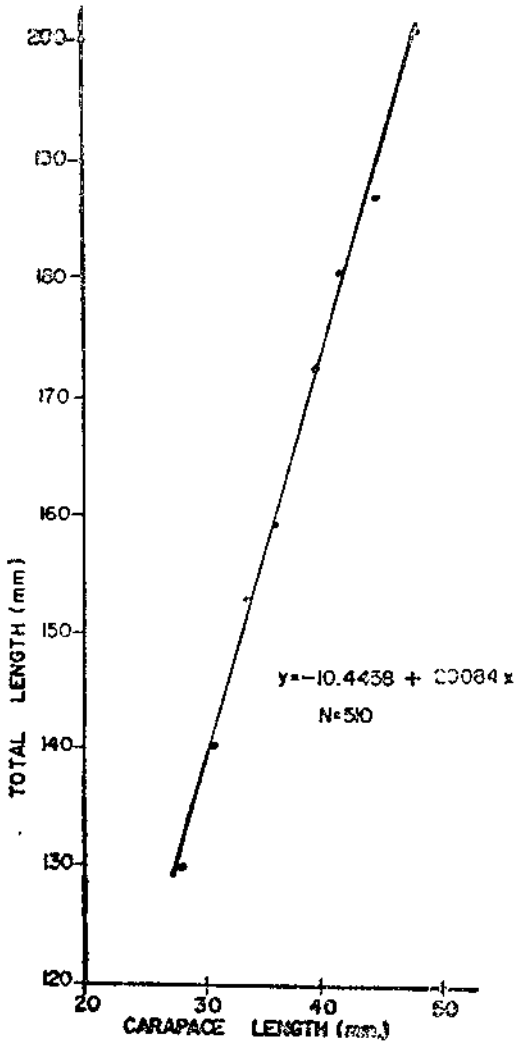

FIG. 1. Relationship between carapace length and total length of male sugpo (*Penaeus monodon* Fabricius) at Panguil Bay.

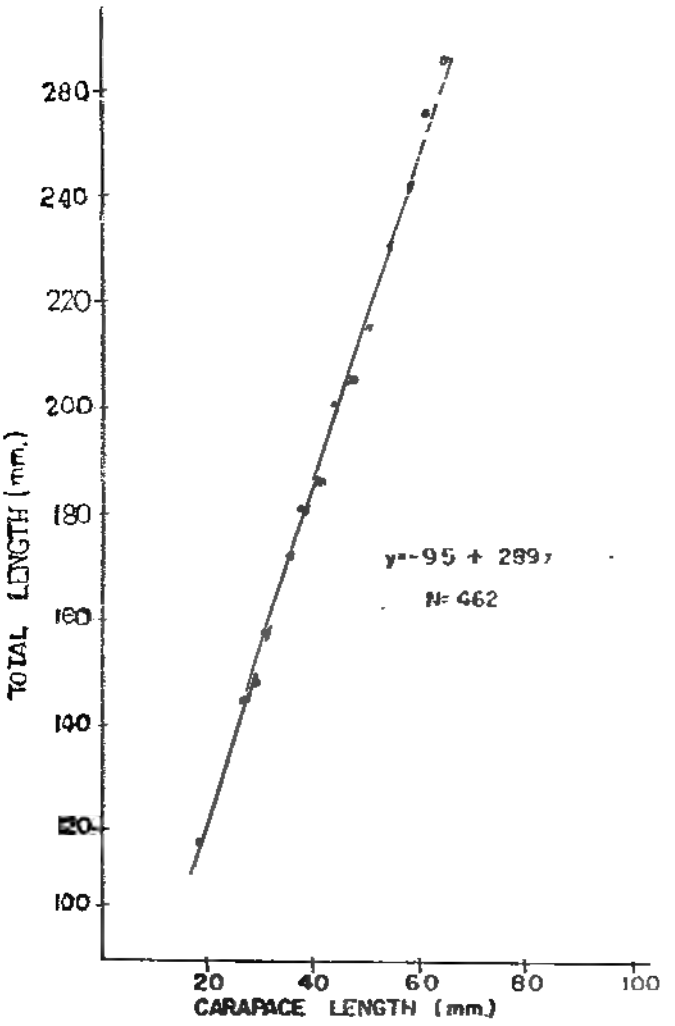

FIG. 2. Relationship between carapace length and total length of female sugpo (*Penaeus monodon* Fabricius) at Panguil Bay.

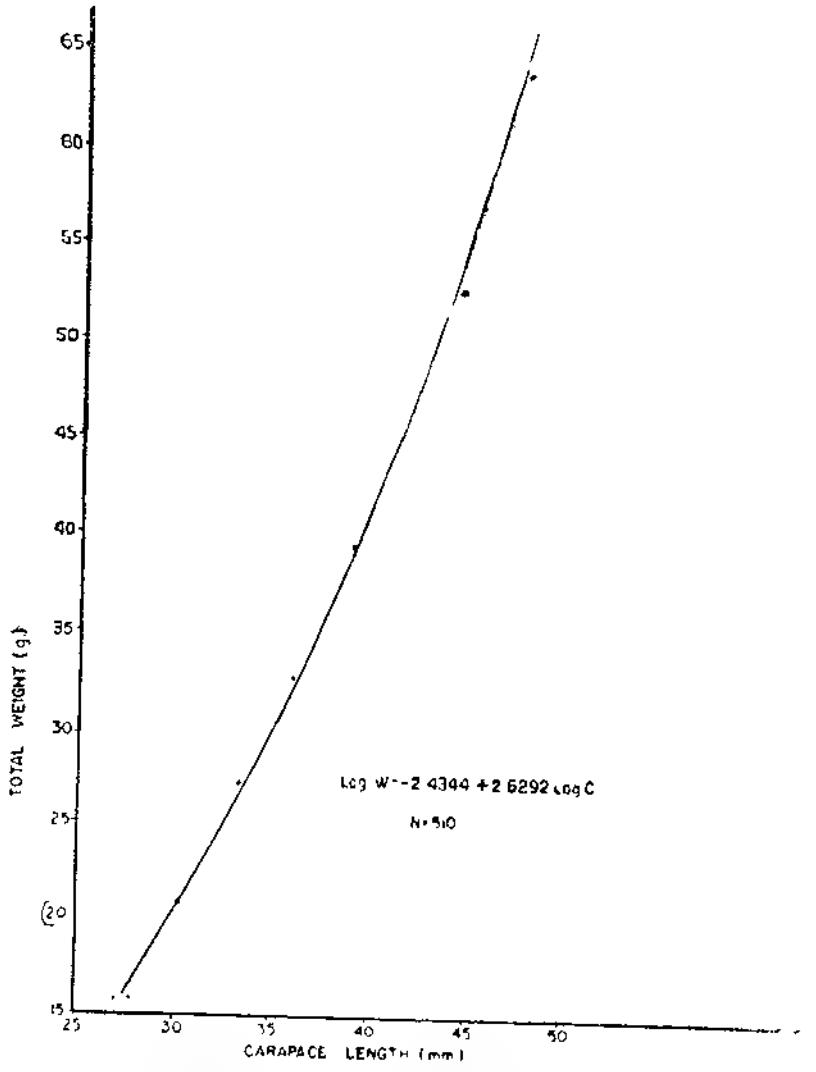

FIG. 3. Relationship between carapace length and total weight of male sugpo (*Penaeus monodon* Fabricius) at Panguil Bay.

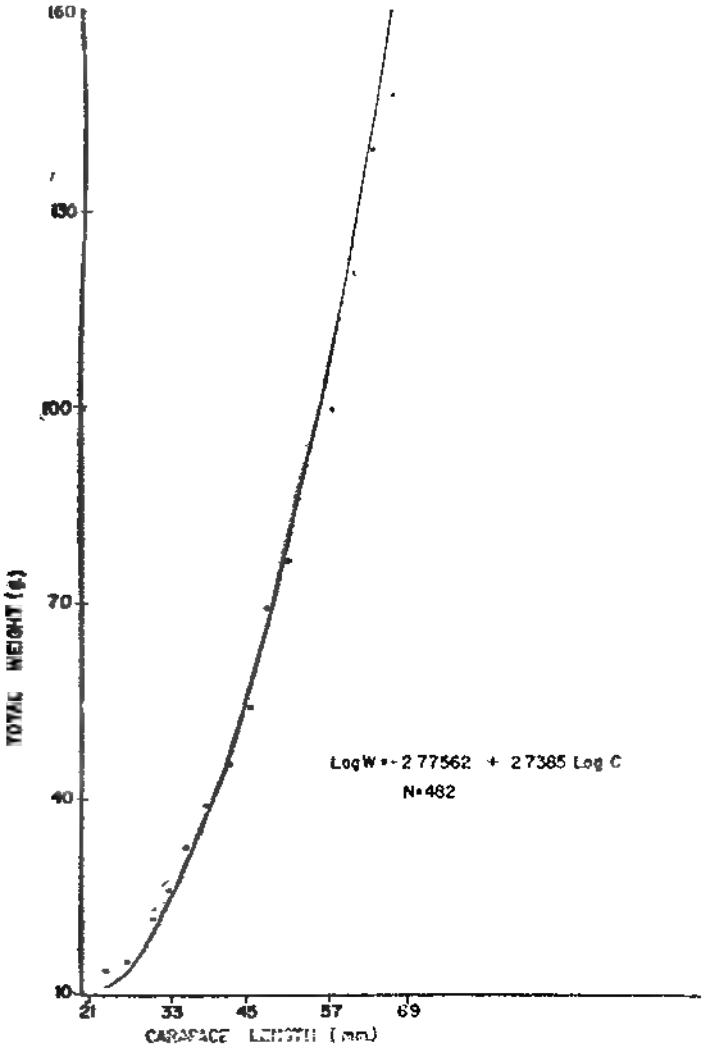

FIG. 4. Relationship between carapace length and total weight of female sugpo (*Penaeus monodon* Fabricius) at Panguil Bay.

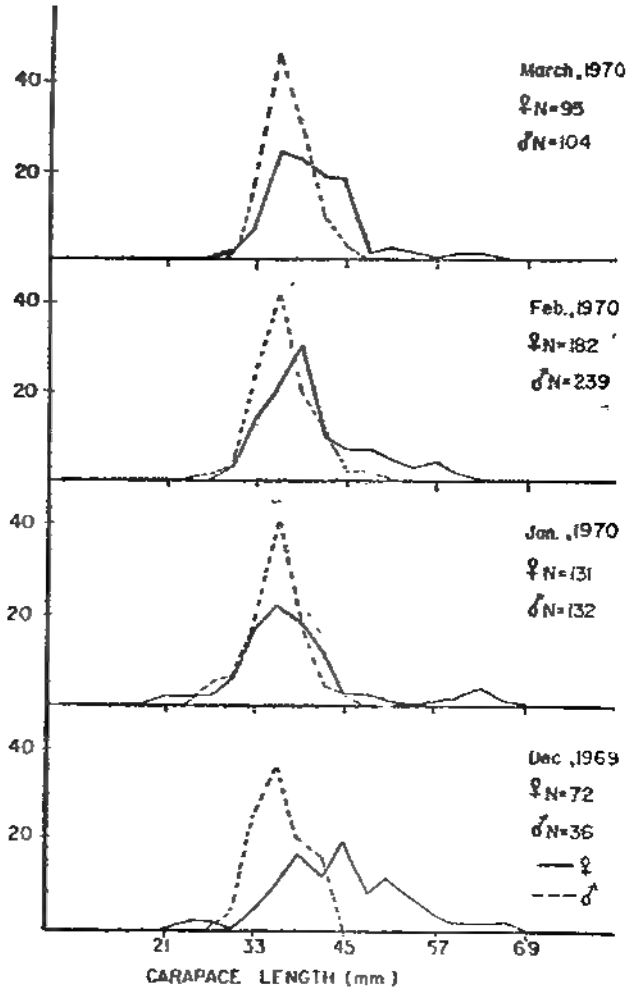

FIG. 5. Carapace length distribution of sugpo (*Penaeus monodon* Fabricius) from December to March 1970 at Panguil Bay.

pointed out by Khandker (1968). From the specimens collected the largest male has a carapace length of 49 mm and the largest female 67 mm. The smallest adult size cannot be considered at this time because the boundary beeween juvenile stage and adult stage is still to be established.

Sex ratio.—According to Tiews (1958) the sex ratio of the commercial shrimp stocks in Manila Bay is more or less balanced. The present data from Panquil Bay tends to differ

from his findings. In our collection from December 1969 to March 1970 the female dominated the male only in December, while males dominated in all the 3 other months. Sampling is continued to complete the year round monthly sex ratio.

Growth rate.—One basis for making an estimate on the growth of crustaceans is their moltings. The growth of prawns is directly related to the molt cycle, since size increases cannot occur while the animal is still encased in its exoskeleton [Schaefer (1968)]. Preliminary findings show that both the male and female specimens placed in aquaria increase their carapace length by 1 mm every 20 days. Tiews (1958) used the Peterson method and estimated the yearly total length increase of female to be some 7 cm or 70 mm and in the male 3–4 cm or 30–40 mm all in the natural habitat. From these estimates, the prawns seem to grow more slowly in the culture tanks, and faster in the open sea. Sex dimorphism based on Tiews' findings first appear at some 50 mm total length: the present work has not yet established this.

Maturation.—Due to apparent inconsistencies as to the number of maturation stages reported by various workers, the present writers temporarily adopted the five maturation stages set by Rao (1968) for four species of Penaeidæ:

1. Immature stage, where the ovaries are thin, translucent, unpigmented and confined to the abdomen;

2. Early maturing stage, where the ovary is increasing in size and the anterior and middle lobes are developing;

3. Late maturing stage, where the ovary is light green and is visible through exoskeleton and the anterior and middle lobes fully developed,

4. The mature stage, where the ovary is dark green and ova larger than in the preceding stage and which is believed to be the last stage of maturity before actual spawning;

5. Spent recovering stage, which is distinguished only from the immature stage by the size of the prawn.

Stages 3 and 4 are found only among female specimens with 60-mm carapace length and above. Fig. 2 shows the general appearance of an enlarged mature ovary of sugpo belonging to Stage 4. Kunju (1968) found that the mature and spent *Solenocera indica* Nataraj have the same size and that in other penaeids spawning follows soon after when once the ovary reaches the mature stage. It is found that the same holds true for *P. monodon.*

Gravid females.—The pregnant or gravid female sugpo are collected from fish corrals popularly known in Panguil Bay as "tower" and from among the hands of gill net fishermen. Prawn fishing is done at night in grounds where the bottom is generally composed of sand and mud, at depths ranging from 3½ fathoms to 26 fathoms.

Unlike the Japanese prawn, the gravid female sugpo does not have the stopper in its thelycum so that it is not easy to determine readily if it has already undergone copulation. Gravid females therefore are selected by examining thoroughly the development of the ovaries through the dorsal epidermal shells. It has been found that females with ovaries which are deep brownish-green in color, thick and well-defined in appearance are apt to lay eggs easily. Under this condition, the spermatophores must have been injected earlier into the body of the females by the males so that the absence of stopper in the thelycum becomes immaterial.

In transporting gravid females from the field to the hatchery, plastic bags measuring 50 × 96 cm, half filled with sea water and charged with oxygen, are used to contain not more than two specimens. The point is to keep them in healthy condition during transit and our experience shows that even after 12 hours, the gravid females spawn normally.

The spawning tanks.—In the MSU Marine Research Laboratory, spawning tanks of different sizes, shapes and materials are used. There are three marine plywood tanks each measuring 2 × 1 × 1 m with a total holding capacity of 6 m^3 of sea water. Another spawning tank is an aquarium measuring 175 × 54 × 50 cm, the front of which is tempered glass 3/16 inch thick.

Sea water is pumped into the tanks during high tide especially when the water is clean and the salinity is high. All the pipes used (for water and for aeration) are poly-vinyl, so with all the valves and other adjustments and accessories. Poly-vinyl is utilized to avoid rust formation which is the result when ordinary G. I. pipes are used. Airstones are used to supply air into the water at the rate of one airstone for every 3 m^2 of bottom utilized.

Spawning.—There are many biological factors which determine the number of pregnant sugpo to be stocked into the breeding tank. However, in the MSU laboratory, 1 m^3 of sea

water for each spawner is utilized. The gravid females arrive before sunset and are immediately transferred into the tanks with water temperature of not lower than 26°C.

Spawning generally takes place at night, between 8 p.m. and 4 a.m., with water salinity ranging from 29 to 33 ppm and water temperature from 27° to 29°C. On the average, each female prawn spawned 15×10^4 fertile eggs. In the case of small and deformed eggs, these were laid in mass and did not become fertilized.

LARVAL DEVELOPMENT

Nauplius.—Sugpo eggs are spherical and isolecithal, with sizes from 0.25 to 0.33 mm in diameter, although majority of them measure 0.27 mm in diameter. Eggs in advanced stage of embryonic development have the appendages prominently developed. The egg membrane is colorless and transparent.

Plate 1, fig. 1 shows the 1st nauplius (N_1) just released from the egg membrane at approximately 12 hours after spawning. The eggs begin to hatch at water temperature ranging from 28° to 29.5°C. The newly hatched nauplii measure from 0.31 to 0.33 mm in body length. Plate 1, figs. 5 and 6 show the 5th nauplius (N_5) that is 0.39 mm in body length and the 6th nauplius (N_6), 0.41 mm body length, respectively. The 6th stage is characterized by elongated body and also by the bilobed posterior end. In addition, the antennule, antenna and mandible are already very distinct.

The sugpo larvæ remain in the nauplius stage for about 48 to 53 hours, molting 6 times at 28°C water temperature. The nauplii swim in all directions and do not require any outside food as they are provided with yolk inside their bodies to last them through this stage up to the first zoea.

Zoea.—The 1st zoea (Z_1) is shown in Plate 2, fig. 1, with body length of 1.2 mm. The larvæ start to take in food as soon as the yolk in their bodies are consumed. Since the zoea are incapable of hunting for their food, it is necessary to provide them with plenty of planktonic food, especially *Skeletonema costatum* within easy reach of their mouths.

Plate 2, fig. 3 shows the 2nd zoea (Z_2) with a total length of 1.74 mm, characterized by the stalked eyes and the rostral and supraorbital spines. Plate 2, fig. 4 shows the 3rd zoea (Z_3) with a total length of 2.55 mm, characterized by a dorsal spine on each of its 5 abdominal segments, a pair of lateral spines on the 5th abdominal segment, 2 pairs of dorsolateral and ventro-lateral spines on the 6th abdominal segment, 6th abdominal segment cut off from telson, and appearance of uropods.

The sugpo larvæ in the zoea stage, if healthy, are active and swim in forward movements drawing threadlike faeces behind their bodies. The food of zoea is composed mainly of mixed forms of diatoms, including *Skeletonema, Melosira, Thalassiosira, Rhizosolenia,* and *Nitzschia.* After 3 moltings within 6 days at 28°C, the zoea metamorphoses into mysis.

Mysis.—Plate 3, fig. 2 shows the 1st mysis (M_1) about 3.5 mm long, with the 3rd maxillipeds and the 5 pairs of pereiopods developed, the uropods fully developed, and the telson still bilobed. The 2nd mysis (M_2), Plate 3, fig. 1, is 3.98 mm long, with pleopods beginning to grow and the 2 lobes of telson starting to join. The 3rd mysis (M_3), Plate 3, fig. 1, is 4.56 mm long; the chelate ends of pereiopods become visible, its pleopods are fully developed but still nonfunctional, and the 2 lobes of telson almost joined.

The sugpo larvæ in the mysis stage appear as if they are minute shrimps, and they swim in vertical position, standing on their heads. The backward dart is accomplished by bending the abdomen, thus enabling them fast movements from time to time. The food of larvæ in this stage are mixed diatoms, minute zooplanktons composed of trochophore, balanus, veliger, copepods and polychaete larvæ. On the last day of M_3, the rearing tank is stocked with brine shrimp (Bs n) nauplii as food of postlarvæ. The mysis stage lasts 4 days and after the third molt follows the postlarval stage.

Postlarvæ.—The first postlarva (P_1), Plate 3, fig. 6, measures about 5 mm in body length. At this stage, the young sugpo molts every day for the first 4 days and every other day subsequently. The larva remains planktonic until the 5th postlarva (P_5), after which it turns benthic, crawling on the bottom and along the walls of the experimental tanks. Important morphological changes noted are the functioning of the 5 pairs of

pleopods for swimming and the use of the pereiopods for grasping and crawling.

The 8th postlarva (P_8) is 6.9 mm long; postlarva 10 (P_{10}) is 8.8 mm; postlarva 12 (P_{12}) is 10 mm; postlarva 13 (P_{13}) is 11 mm; postlarva 18 (P_{18}) is 15 mm and postlarva 39 (P_{39}) varies in size from 33 to 46 mm body length and from 8 to 12 mm carapace length.

The postlarvæ of sugpo become carnivorous, changing from their omnivorous food habit of the mysis stage. They are given mostly brine shrimp nauplii during the first 4 days of the postlarval stage. Shigeno (1970) observed that *P. japonicus* in its early postlarval stage devours 46–84 brine shrimp nauplii in 24 hours. From the 5th postlarva (P_5) the young sugpo are fed with minced shell meat.

Postlarva 25 (P_{25}) measures about 25 mm in body length At this stage, the postlarvæ are harvested from the rearing tanks and are now ready for stocking in ponds. More than 10,000 P_{25} sugpo fry were produced from the eggs of one mother prawn.

ACKNOWLEDGMENT

Our heartfelt gratitude is due to Rev. Father James McKeough, chairman, Department of Biology, Xavier University, Cagayan de Oro City, for his kind permission to use their microphotography equipment; to the MSU University Research Center and the NSDB for financial assistance which has made this research possible; to the Technical Cooperation Program of the Government of Japan and the Foreign Affairs Department, Republic of the Philippines, for sponsoring the observations on prawn culture in Japan by the senior author; to Congressman Ali Dimaporo for offering the use of some facilities in his fishponds; to the Mindanao Development Authority for its interest in putting up pilot commercial sugpo ponds wherein the results of our research may be utilized; to fishpond owners, shrimps fishermen, shrimp middlemen and vendors whose cooperation in our collection of specimens we highly appreciate; and to all those who in one way or another made possible the completion of this report.

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TABLE 1.—*Comparative data showing the relationship among carapace length, total length and total weight, both observed and calculated, of the male and female sugpo (Penaeus monodon Fabricius).*

Number	C. L. Size group	Mid PTS	Frequency		Mean C. L.		Mean T. L.		Mean T. W.		Calculated T. L.		Calculated T. W.	
			M	F	M	F	M	F	M	F	M	F	M	F
					mm	mm	mm	mm	gm	gm	mm	mm	gm	gm
1	23 2[illegible]	24		2	[illegible]	23 50	[illegible]	116 50	[illegible]	13 70	[illegible]	114 18	[illegible]	11 2642
2	26-28	27	7	3	27 86	26 66	130 00	123 00	15 827	14 [illegible]	131 697	125 12	16 [illegible]993	13 4369
3	29 31	30	18	17	30 39	30 4[illegible]	140 58	143 38	20 770	[illegible].86	140 406	138 30	20 6865	19 [illegible]14
4	[illegible] 34	33	108	58	33 42	33 [illegible]9	152 94	148 42	27 1[illegible]2	25 69	150 829	148 06	26 3[illegible]04	24 6[illegible]3
5	3[illegible] 37	36	210	103	36 04	36 02	1[illegible]9 84	158 29	32 66[illegible]	31 69	159 833	157 50	3[illegible] 8105	3[illegible] 7011
6	38 40	39	108	114	[illegible]9 26	39 05	170 90	171 35	39 268	38 77	170 905	168 0[illegible]	38 [illegible]088	38 [illegible] 27
7	41 43	42	1[illegible]	59	41 [illegible]3	41 50	180 61	179 79	47 494	4[illegible] 59	178 719	177 85	4[illegible] 3827	46 [illegible]20
8	44 46	45	10	48	44 [illegible]	41 98	186 94	18[illegible] 65	[illegible]2 82[illegible]	54 36	189 127	188 [illegible]1	[illegible]1 4284	[illegible]6 2600
9	47 49	48	4	22	48 00	48 04	200 62	198 8[illegible]	68 [illegible]90	69 36	200 955	199 16	6[illegible] 72[illegible]	67 6239
10	[illegible]0 2	51		19	[illegible]	50 63	[illegible]	204 15	[illegible]	76 24	[illegible]	208 06	[illegible]	77 6668
11	[illegible] [illegible]	[illegible]4		11	[illegible]	53 [illegible]3	[illegible]	214 68	[illegible]	90 78	[illegible]	218 78	[illegible]	91 4[illegible]95
12	[illegible]6 58	[illegible]7		9	[illegible]	57 00	[illegible]	228 53	[illegible]	90 48	[illegible]	230 10	[illegible]	107.091
13	[illegible]9 61	60		7	[illegible]	60 43	[illegible]	240.75	[illegible]	120 23	[illegible]	241.97	[illegible]	125.777
14	62 64	63	[illegible]	8	[illegible]	63.10	[illegible]	254 75	[illegible]	1[illegible] 84	[illegible]	251 62	[illegible]	142 856
15	6[illegible]-67	66	[illegible]	2	[illegible]	66.00	[illegible]	262 50	[illegible]	116.97	[illegible]	261 24	[illegible]	159.956

TOTAL: Male, 510; female, 482.

TABLE 2.—*Characteristics of the larval stages of sugpo (Penaeus monodon Fabricius) : Nauplius stages.*

Stage	Length	1st Antennæ	2nd Antennæ	Mandible	Posterior end	Median eye and labrum	Rudimentary structures
1st Nauplius (N$_1$)	*mm* 0 31	Uniramous; 6 setae, 2 long and 1 short at tip and 1 long and 2 short at sides	Biramous, slightly longer than endopodite, Exopodite, 5 setae: 2 long at tip and 3 long at sides, Endopodite, 3 setae: 2 long at tip and 1 short at side	Biramous shorter than antennæ, Endopodite slightly longer than exopodite, 3 long terminal setæ, Endopodite, 3 long terminal setæ	Flat end, Spine formula: 1 + 1, spines slightly flexed dorsally.	Median eye and labrum present	None
2nd Nauplius (N$_2$)	0 33	Uniramous, 6 setae: 2 long and 1 short at tip and 1 long and 2 short at sides, setae plumose	Biramous, Exopodite 6 setae: 2 long and 1 short at tip and 3 long at sides; Endopodite, 4 setae: 2 long and 1 short at tip and 1 short at side; long setæ plumose	Biramous, Exopodite 3 long terminal setæ, Endopodite, 3 long terminal setæ Setæ plumose.	Flat end, spine formula: 1 + 1, spine surrounded by short flat sharp spinules	Median eye and labrum present, A pair of indistinct frontal organs at anterior end of body.	Rudimentary structures at ventral side below labrum faintly visible.
3rd Nauplius (N$_3$)	0 36	Uniramous, 5 setae, 3 long at tip and 2 long at sides, long setae plumose	Biramous, Exopodite, 7 setae: 3 long at tip and 3 long at tip and 1 short at side, Endopodite, 6 setae: 3 long at tip and 3 short at sides Long setæ, plumose, segmentation of exopodite and protopodite faintly visible.	Biramous, Exopodite 3 long terminal setæ, Endopodite 3 long terminal setæ, long setæ plumose, inner side of protopodite slightly swollen	Bifurcate, Spine formula: 3 + 3, long center spines plumose.	Median eye and labrum present, Labrum faintly appear below labrum	Rudiments of 2 pairs of maxillæ and 1st 2 pairs of maxillipeds appear below labrum

TABLE 2.—*Characteristics of the larval stages of sugpo (Penaeus monodon Fabricius): Nauplius stages*—Continued.

Stage	Length	1st Antennæ	2nd Antennæ	Mandible	Posterior end	Median eye and labrum	Rudimentary structures
4th Nauplius (N$_4$)	0.38	Uniramous; 5 setae: 3 long at tip and 2 short at sides; setae plumose.	Biramous; Exopodite 7 setae: 2 long and 2 short at tip and 3 long at side, 6 distinct segments; Endopodite, 5 setae: 3 long at tip and 2 short at side; protopodite, 3 distinct segments; long setae plumose.	Biramous; Exopodite, 3 long terminal setae; Endopodite, 3 long terminal setae; Exopodite and endopodite distinctly separated from protopodite; Ventral side of protopodite swell; Long setae plumose	Bifurcate; Spine formula 4 + 4, long center spines plu-[illegible]	Median eye and labrum still persist.	Rudiments of 2 pairs of maxillae and 1st 2 pairs of maxillipeds biramous
5th Nauplius (N$_5$)	mm 0.39	Uniramous: 6–7 setae: 2 long and 1 short at tip and 3–4 short at sides; 2–3 tiny spines at sides; Numerous faint articulations of inner end, setae plumose.	Biramous, Exopodite, 9 setae: 3 long and 1 short at tip, 4 long and 1 short at sides; 8 segments. Endopodite, 6 setae: 3 long and 1 short at tip and 2 short at sides; Protopodite, 3 segments; long setae plumose.	Biramous; Exopodite, 3 long terminal setae; Endopodite, 3 long terminal setae; Exopodite and endopodite separated from protopodite; Semispherical masticatory process formed at protopodite; Setae plumose.	Bifurcate; Spine formula 6 + 6; longer spines plumose	Median eye, labrum and labrum still persist.	Rudiments of 2 pairs of maxillae and 1st 2 pairs of maxillipeds elongated; Posterior margin of shell fold aligns with the rudiment of 1st maxilla; Slight concavity of anterior end.

TABLE 2.—*Characteristics of the larval stages of sugpo (Penaeus monodon Fabricius): Nauplius stages*—Continued.

5th Nauplius (N5)	0.41	Uniramous 6-7 setae: 2 long and 2 short at tip and 2-3 short at sides, 2-3 tiny spines at sides, 9-11 short basal segments and 1 long terminal segment, long setae plumose	Biramous, Exopodite 10 setae: 3 long and 1 short at tip and 4 long and 2 short at sides, 9 segments, Endopodite 6-7 setae 4 long at tip and 2-3 short at sides, 4 podomeres, 3 segments, Long setae plumose	Biramous, Exopodite and endopodite each setae 3 long plumose terminal setae, buttress, practically empty, almost totally ineffective for swimming, Rudiment of a short, unarticulated and unsetted marginal exite under exite	Bifurcate, median notch deepened, Spine formula 7+7 Longer spines plumose	Medium eye, labrum and labrum with 1 pair set, a faint mark at mid posterior margin of labrum	Rudiments of 2 pairs of maxillipeds and 1st 2 pairs of maxillipeds much elongated and with tiny sets; Posterior mark of ashe, food cleft and rudiment of 1st maxilliped, slight heavy of anterior end
6th Nauplius (N6)							

WORK ORDER NUMBER	SPECIALTY NUMBER	TIME		QUANTITY PRODUCED	HOURS		
		STARTED	STOPPED OR FINISHED		TOTAL	CHARGEABLE	NON-CHARGEABLE

TABLE 3.—*Characteristics of larval stages of sugpo (Penaeus monodon Fabricius): Zoea stages.*

Stage	Length	Carapace and rostrum	1st Antenna	2nd Antenna	Mandible	1st Maxilla	2nd Maxilla	1st Maxillipede	2nd Maxillipede	Abdomen and telson
	mm									
1st Zoea Z_1	1.2	Carapace irregular octagon in shape; Nauplius eye still present 6 thorax somites present.	3 segments: 1st segment composed of 5 still smaller segments; 3 long setae at tip and 3 long and 3 short at sides.	Protopodite: 3 segments: Exopodite: 7–9 segments 5 long setae at tip, 6 long and 1-2 short setae at sides; Endopodite: 2 segments, 4 long and 1 short setae at tip, 2 long and 2 short setae at sides.	Exopodite and Endopodite disappear. Masticatory portion appears.	Protopodite: 2 lobes or endites at inner side, 7 setae at 1st endite 5 setae at second; Exopodite: small and spherical, 4 setae; Endopodite: 3 segments, 3 setae at 1st segment 5 setae at 2nd and 3rd segments	Protopodite: 5 small lobes or endites at inner side; 5–7 setae at 1st endite, setae at other endopodites; Exopodite: spherical, 5 setae, Endopodite: 4 segments, 3 long setae at tip of last segment, 2 setae each on sides of 1st 3rd segment.	Protopodite: 2 segments, 4–6 setae on 1st segment 12–15 setae on 2nd segment; Exopodite: shorter than endopodite, 3 setae at endopodite, tip, 4 at outer sides, endopodite: 4 segments 5 setae at tip of last segment 2 [illegible] setae for each of sides of 1st 3rd segments	Protopodite 2 faint segments with 4–5 setae at sides; at sides; Exopodite: slightly shorter than endopodite, 3 setae at tip [illegible] setae at sides; Endopodite: 4 segments, 4 [illegible] setae at tip of segment, 1- [illegible] on each of 1st-3rd segments	Abdominal somites absent; 2 lobes at telson separated by semispherical notch, Spine formula 7 + 7.

TABLE 8.—*Characteristics of larval stages of sugpo (Penaeus monodon Fabricius): Zoea stages*—Continued.

2nd Zoea (Z_2)	mm 1.74	Long rostrum, slightly curved at its tip; A pair of supraorbital spines bearing 2 tiny spines on their tips; A pair of compound eyes; Rudiments of 3rd maxillipede and 5 pairs of thoracic appendages appear.	Same as zoea I	Same as Z_1	Same as Z_1	Same as Z_1	Same as Z_1	Same as Z_1	Same as Z_1	5 abdominal somites distinct boundary between 6th somite; and telson not discernible; Spine formula: 7+7.

TABLE 3.—*Characteristics of larval stages of suggo (Penaeus monodon Fabricius): Zoea stages*—Continued.

Stage	Length	Carapace and rostrum	1st Antenna	2nd Antenna	Mandible	1st Maxilla	2nd Maxilla	1st Maxilliped	2nd Maxilliped	Abdomen and telson
3rd zoea (Z_3)	2.5	Small spines at tips of supraorbital spines disappear. Rudiments of 6 pairs of thoracic appendages slightly developed as endopodites. 3rd maxilliped slightly elongated with 3 short setae at tips.	5 small basal segments combined into 1 wide segment.	Same as Z_1	Molar process widens.	2 lobes or endite protruded.	Same as Z_1	Same as Z_1	Same as Z_1	A small median dorsal spine each from 1st to 5th abdominal somites. A pair of posterolateral spines on 5th somite; 6th somite cut off from telson with a pair of dorsal lateral spine and another pair of ventrolateral spine. Uropod appears biramous; Exopodite slightly longer than endopodite with 6–7 setae at tip and sides. Spine formula at telson: 8+8

TABLE 4.—*Characteristics of larval stages of sugpo (Penaeus monodon Fabricius): Mysis stages.*

Stage	Length	Carapace and rostrum	1st and 2nd Antennæ	Mandible	1st and 2nd Maxillæ	1st, 2nd, 3rd Maxillipeds	Pereiopods	Pleopods	Abdominal segments	Telson and uropoda
1st Mysis (M1)	mm 3 5	Supra-orbital spines shrunk. A pair of antero-lateral spines appear below the middle of anterior margin of carapace, A pair of hepatic spines appear behind eye and set back from anterior margin of carapace, Rostrum slightly longer than eyestalk, with no tooth or dorsal side	1st Antenna 3 segments, several setæ; 1st segment longest, edge of tooth outside base of 1st segment One spine at ventral side of 1st segment 2 branches at distal end of 3rd segment, outer branch about twice as long as inner, with 6 simple setæ, inner branch with 2 simple setæ at end. *2nd Antenna* Protopodite 2 segments, endopodite and exopodite disappear	Number of small teeth increases, A small projection appears at upper margin of peduncle	1st Maxilla: 2nd lobe of protopodite protrudes, *2nd Maxilla* Exopodite much developed than in 3rd zoea, 10 long plumose setæ	1st Maxilliped: Same as in the zoea stages *2nd Maxilliped* Same as in the zoea stages *3rd Maxilliped*, Protopodite 2 segments, Endopodite 5 segments, Exopodite, none, Exopodite 5-6 setæ at tip, Endopodite 1 3 setæ on sides of 1st 4th segments, 1 5 setæ at tip of 5th segment	Protopodite 2 segments Exopodite longer than endopodite, Exopodite 4 long setæ at end, 3 long setæ at sides, Endopodite 3 4 long simple setæ at tips	Pleopods appear as buds at ventral side of abdomen.	Spines on 1st and 2nd segments disappear, A long spine appears on median line of 6th segment at postero-dorsal margin, Lateral spines at 6th segment shrink A spine appears between 6th segment and telson at mid postero-ventral margin	Telson Height of tip of notch between lateral 1st and 2nd spines, Spines formula, 8 + 8 Uropoda Well developed A spine appears at outside edge of protopodite, Exopodite slightly longer than endopodite, shorter than telson, small spine on outer distal margin; 16-17 plumose setæ at outer and lateral margin; Endopodite: 15-16 plumose setæ at distal and lateral margin.

TABLE 4.—*Characteristics of larval stages of sugpo (Penaeus monodon Fabricius): Mysis stages—Continued.*

Stage	Length	Carapace and rostrum	1st and 2nd Antennæ	Mandible	1st and 2nd Maxillæ	1st, 2nd, 3rd Maxillipeds	Pereiopods	Pleopods	Abdominal segments	Telson and uropod
			pear, Exopodite attained, 10-15 long plumose setæ at tip and sides Endopodite shorter than exopodite, rod like shape at tip and 3-5 simple setæ on side							
2nd Mysis (M2)	mm 3.98	Carapace same as in 1st Mysis. Rostrum slightly longer than eyestalk. One tooth appears at dorsal side of rostrum	1st Antenna: the inner branch at the end of the 3rd segment about 2/3 that of the outer 2nd Antenna: small size exopoeters at the 2nd segment of the protopodite Exopodite terminates with a short spine at the outer tip; has 17-18 setæ on the tip and on the side. Endopodite	A small protrusion at the upper margin of the peduncle grows longer.	1st Maxilla same as 1st Mysis 2nd Maxilla 16 long setæ around the exopodite	1st Maxilliped: Same as 1st mysis 2nd Maxilliped Same as 1st Mysis 3rd Maxilliped Same as 1st Mysis	The endopodites of each appendage becomes longer than ½ exopodites, first 3 pairs being segmented into 4 and the remaining 2 pairs into 5	Pleopods are slightly more pronounced and elongated.	3rd and 4th segments slightly curved a hump dorsally.	A Telson: The tip of telson is bow same level as 2nd spine. Spine formula: 8, 8 B Uropod. Exopodite bears 19-21 setæ at the distal and lateral margin. Endopodite has 17-19 setæ.

TABLE 4.—*Characteristics of larval stages of suggo (Penaeus monodon Fabricius): Mysis stages—Continued.*

3rd Mysis (M3)	4 56	Carapace, same as 2rd mysis. Rostrum: almost equal to eyestalk, one dorsal tooth.	as 2 3 as long as the exopodite, and setæ are lacking. 1st Antenna: otolith visible at base, inner branch at end of 3rd segment longer or as long as the outer branch. Outer branch 2 faint segments; 6 simple setæ at tip and 3 on the sides; inner branch 3 faint segments, 4 simple setæ at tip. 2nd Antenna: Exopodite, 21 23 setæ, endopodite, 4 faint segments, 2 3 tiny setæ at end	2 segments on small protrusion	1st Max illa: Exopodite disappears 2nd Maxilla: 19 20 long setæ at exopodite	1st Maxilliped: Same as in 2nd Mysis 2nd Maxilliped: Endopodite segments, numerous setæ. 3rd Maxilliped: Same as in 2nd Mysis	Exopodite: 2rd Mysis Endopodite 1st 3 pairs of endopodite, 7 segments. Ends of 1st 3 pairs chelate	Elongated. 2 segments, 2 3 tiny setæ at tips.	Lateral spines at 5th somite much reduced. Other spines the same as in 2nd mysis.	Telson: Posterior margin almost flat. Spine formula, 8 + 8. Uropod Exopodite with plumose; 22 24 setæ at distal and lateral margin. Endopodite with 20 22 plumose setæ.

TABLE 5.—*Characteristics of larval stages of sugpo (Penaeus monodon Fabricius): Post-larva.*

Stage	Length	Carapace and rostrum	1st and 2nd Antennae	Mandible	1st and 2nd Maxillae	1st, 2nd, 3rd Maxillipeds	Pereiopods	Pleopods	Abdominal segments	Telson and uropod
1st Post-larva (P1)	mm 4.84	Carapace same as in 3rd mysis, Rostrum about ¼ longer than eye stalks, with 2 dorsal teeth.	1st Antenna statolith visible at base, a sharp ventral spine persists. 2nd Antenna same as in 3rd mysis.	Teeth at grasping side lessened in number but sharpened	1st Maxilla: 2 lobes at peduncle sharpened setae increased, endopodite degenerated. 2nd Maxilla: 4 lobes 2 at inner side of peduncle, 4th lobe the largest, Number of setae decreased; Endopodite greatly degenerated.	1st Maxilliped: 2 lobes inner side of peduncle, 1st lobe subdivided into 2 more lobes. Exopodite slightly larger and flat, Endopodite unsegmented and degenerated. 2nd Maxilliped: 3 setae inner side of 1st joint at peduncle, 7 setae on the second joint. Exopodite without setae and degenerated. Endopodite, 4 segments. 3rd Maxilliped: 1st joint of peduncle without setae, 2nd joint 2 [illegible] setae. Exopodite without setae, and degenerated. Endopodite, 5 joints.	1st 2nd and 3rd Pereiopods Peduncle with 2 joints while endopodite with 5 joints 4th and 5th joints with few short setae, each form a chela. Exopodite tips [illegible] with sharp short setae growing densely around chela without teeth.	Pleopods become functional with 4-6 long setae.	Spines same as in M3	Telson spine formula 8+4 small [illegible] present on median part of [illegible] Uropod. Endopodite with 22-23 plumose setae. Exopodite 21-22 plumose setae.

ILLUSTRATIONS

PLATE 1

(Nauplius stages showing parts in detail.)

FIG. 1. First nauplius, ventral view.
2. Second nauplius, ventral view (Inset: seta enlarged.)
3. Third nauplius, ventral view.
4. Fourth nauplius, ventral view.
5. Fifth nauplius, ventral view.
6. Sixth nauplius, ventral view.

PLATE 2

(Mysis and postlarva stages showing parts in details.)

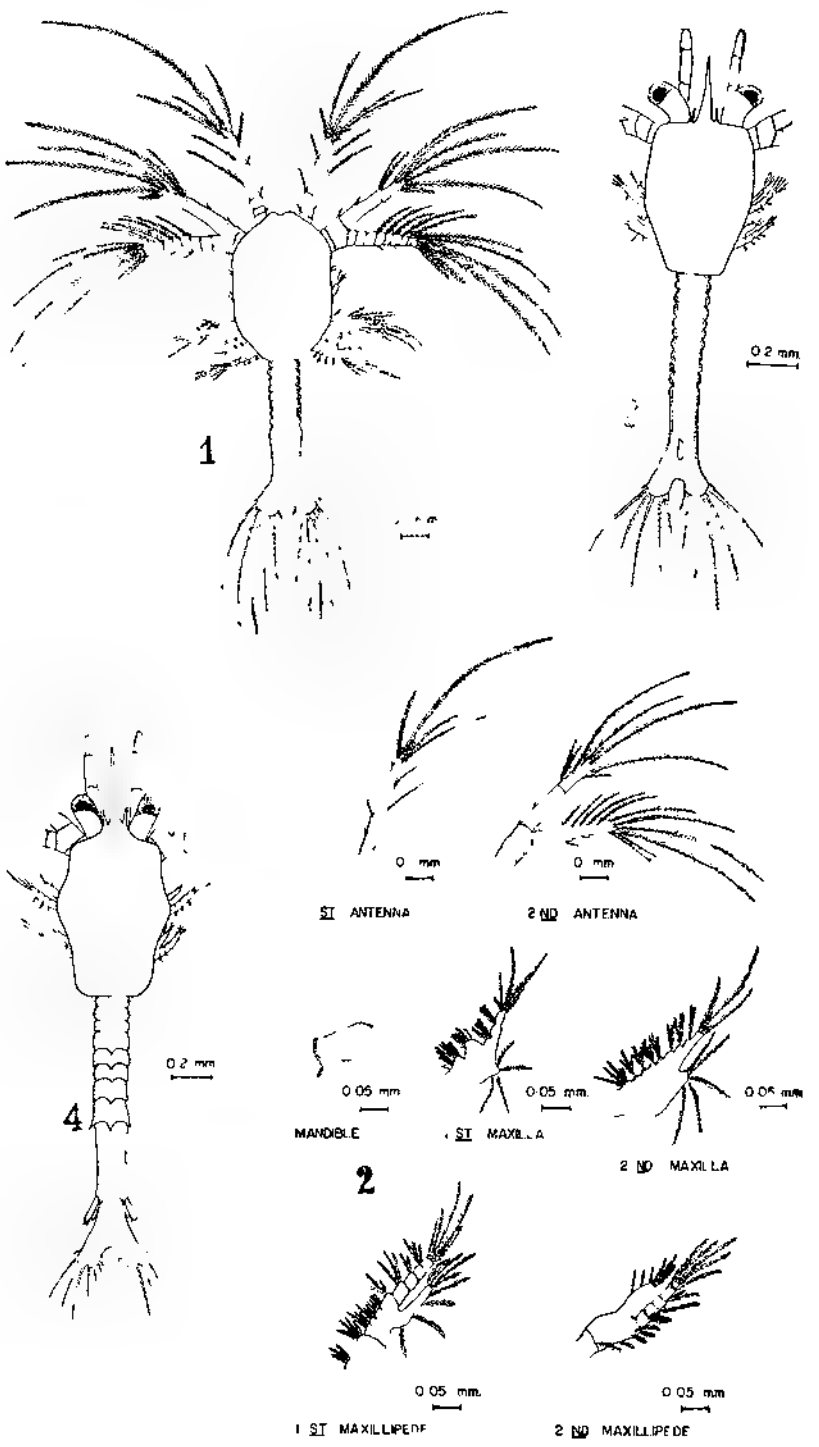
FIG. 1. First zoea, dorsal view.
2. Main parts of first zoea.
3. Second zoea, dorsal view.
4. Third zoea, dorsal view.

PLATE 3

(Mysis stages showing parts in details.)

FIG. 1. The different substages of mysis.
2. First mysis, ventral view.
3. Main parts of first mysis.
4. Main parts of second mysis.
5. Parts of third mysis.
6. First postlarva, ventral view.

PLATE 1.


1
0.2 mm
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MANDIBLE
1 ST MAXILLA
2 ND MAXILLA
2
0.05 mm
0.05 mm
1 ST MAXILLIPEDE
2 ND MAXILLIPEDE

PLATE 2.

PLATE 3.

CLINICAL EVALUATION OF NIST-PRODUCED ALLERGENIC EXTRACTS, I

SKIN TESTING WITH POLLEN EXTRACTS (GRASSES AND WEEDS)

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Since 1906, when von Pirquet and Schick (1951) described the allergic reaction and introduced the word "allergy" into medical literature, research along this line has made remarkable progress. This has been true for countries in the western hemisphere, notably in North America and Europe, where extensive investigations concerning the relation of locally existing airborne pollens to prevalent allergic respiratory diseases by skin testing methods have been done. [Vaughan and Black (1954a), Duchaine (1959).] Other countries like Israel, India, and Japan have also made significant contributions in recent years. [Horiguchi and Saito (1964), Kantor *et al* (1966), Matsumara *et al* (1969), Shivpuri and Dua (1963).] However, in the Philippines, this has been a much neglected problem and there is hardly any literature available on this subject.

A more intensive study of this particular aspect of allergy should be made in our country for several reasons. Allergic respiratory diseases in the form of rhinitis and asthma occur very frequently here as shown by actual clinic experiences and hospital records. [Castillo-Ochoa and Agbayani (1968).] However, there are still no specific figures available on the prevalence rate of such diseases in our population. Since airborne pollens constitute an important cause of respiratory allergies, observations should be made on our own local pollens because of the differences in certain vegetation between the countries where most of the studies have been made and ours. Such information is necessary for the formation of a correct etiologic diagnosis in patients afflicted with allergies especially

of the respiratory type, if these individuals are to get the full benefit of specific treatment. Also, with wider and rapid population movement as a result of more modern methods of travel and increasing worldwide emphasis on tourism, knowledge of inhalant allergens prevailing in a given locality becomes highly important from the standpoint of exposure and consequent medical management of the allergic individual who wants to travel or change his place of residence.

Realizing the need for such investigative work, the Allergy Unit of the Medical Research Center, National Institute of Science and Technology, has begun a series of studies with this specific problem in mind. The following work being presented was undertaken with the following objectives: (*1*) The determination of the indigenous grasses and weeds in Manila and its immediate environs which are important in the causation of prevalent respiratory allergic diseases by skin-testing methods on a larger number of individuals with allergic rhinitis and/or allergic asthma, using NIST-prepared pollen extracts from these plants; (2) the determination of the suitability of these extracts for diagnostic purposes from the standpoint of efficacy, potency, and safety.

MATERIALS AND METHODS

Pollen extracts.—Extracts from the pollens of 17 grass species and 5 species of weeds which were found to be widely distributed and abundant in Manila and its immediate surrounding areas were used as test materials. These consisted of the following:

Grasses (uncultivated): Bermuda grass [*Cynodon dactylon* (L.) Pers.], yard grass [*Eleusine indica* (L.) Gaertn.], *talahib* [*Saccharum spontaneum* (L.) subsp. *indicum* Hack.], para grass [*Brachiaria mutica* (Forssk.) Stapf.], foxtail [*Pennisetum polystachyum* (L.) Schultz.], Java grass (*Polytrias praemorsa* Hack.), Guinea grass (*Panicum maximum* Jacq.), *kogon* [*Imperata cylindrica* (L.) Beauv.], carabao grass (*Paspalum conjugatum* Berg.), crab grass (*Digitaria* species), *batad-batadan* [*Sorghum halepense* (L.) Pers.], *alabang-x* [*Dicanthium aristatum* (Poir.) C. E. Hubb.], natal grass [*Rynchelytrum repens* (Willd.) C. E. Hubb.], *amor-seco* (*Andropogon aciculatus* Retz.).

Grasses (cultivated): rice (*Oryza sativa* Linn.), maiz (*Zea mays* Linn.), sugarcane (*Saccharum officinarum* Linn.).

Weeds: *uraí* (*Amaranthus spinosus* Linn.), *mutha* (*Cyperus rotundus* Linn.), tridax (*Tridax procumbens* Linn.), *makahiya* (*Mimosa pudica* Linn.), sunflower (*Tithonia diversifolia* A. Gray).

Of the aforementioned anemophilous grasses, Payawal and Laserna (1965) found that the most widely distributed are Bermuda grass, yard grass, and *talahib* while *kogon*, para grass, foxtail, carabao grass, Java grass, and Guinea grass are moderately abundant in Manila and its suburban areas.

The concentrated extracts were prepared according to the method described by Laserna and Manalo (1966). Briefly, this was as follows: 4 grams of pollen were macerated with enough Coca's solvent,[1] toluol placed on top, and the mixture stored at room temperature for 3 days. This was then decanted and made up to 100 cc with Coca's solvent, passed through a Berkefeld filter, and sterility tests done to ensure freedom from contaminating microorganisms. The extract was standardized according to protein nitrogen content. Nitrogen was determined in the protein fraction by the micro-Kjeldahl titrimetric method. The concentrate material containing 5,000 PNU/ml was used for the intradermal tests. Evans buffered saline solution[2] was used as diluent for the intradermal test solutions.

Test subjects.—One hundred twenty individuals with allergic rhinitis and/or allergic asthma were used as test subjects. Most of them came from Manila and the nearby suburbs. All were Filipinos except for two Caucasians who had been continuously residing in the Philippines for the last 6 to 10 years. There were 68 males and 52 females with ages ranging from 4 5/12 years to 67 1/12 years. Duration of allergic respiratory disease before inclusion into the study ranged from 3 weeks to 51

[1] Alkaline extracting fluid (Coca) [Vaughan and Black (1954b)].

NaCl	5.00 g
$NaHCO_3$	2.75 g
Phenol	4.00 cc

Distilled water to make 1000 cc.

Stock solution No. I

[2] Buffered saline (Evans) [Vaughan and Black (1954b)].

NaCl	50.00 g
KH_2PO_4	3.63 g
$Na_2HPO_4 12H_2O$	14.31 g

Distilled water up to 1000 cc

Stock solution No. II

Carbolic acid, 4 per cent

The extracting fluid is made by mixing 1 part of Solution I, 1 part of Solution II, and 8 parts of distilled water.

years. Average duration of allergic rhinitis was 10 $^{1}/_{12}$ years and asthma was 9 $^{8}/_{12}$ years. About one-third of the subjects (31.6 per cent) had an associated history of urticaria or other forms of allergic dermatitis; 103 patients (85 per cent) gave a positive family history of allergy.

Diagnosis of allergy was made after a positive clinical history and the presence of past and/or concomittant allergic symptoms and signs supported by results from physical and routine laboratory examinations including routine blood count, urinalysis and feces examination (the latter two when indicated). Blood smears and nasal secretions were also examined for eosinophil content.

Skin test methods.—Direct skin testing was done, using the scratch and the intradermal methods.

Scratch test. After cleansing the flexor surface of the forearm or the back with alcohol, test sites at 1-inch intervals or more were marked with a skin pencil. Scratches from $^{1}/_{8}$ to $^{1}/_{4}$ inch long were then made directly opposite the marked sites with a sterile skin needle. A drop of concentrate extract was then placed on each site. A control test using the extracting solution was also made at the same time. All these tests were made at 1 sitting. Readings were taken after 20 to 30 minutes following the criteria of Vaughan and Black (1954 c):

Negative—No reaction or as determined by the control site or the general average of nonreacting scratches.

Positive—1 + if the wheal is twice that of the control reaction.
2 + larger wheal without pseudopod formation
3 to 4 + reactions with pseudopods which are larger in size and extent.

Intradermal test. After cleansing the flexor surface of the forearm with alcohol, 0.01 to 0.02 ml of the allergenic material was injected intracutaneously from a tuberculin syringe, making a pin-head sized wheal. A vertical row of tests, numbering from 6 to 7 about 2 inches apart were made on each arm. Starting with extracts of each pollen allergen containing 10 PNU/ml of solution, this was followed by a solution containing 100 PNU/ml of the same allergen if the results from the first series of tests were negative.

Readings were made after 5 to 15 minutes following the previously mentioned criteria of Vaughan and Black. The positive reactions were then classified according to the criteria of Cooke, Vander Veer and Bernard [Vaughan and Black (1954d)] into: (*1*) Very sensitive or strong reaction if positive to a dilution of 10 PNU/cc of solution: (*2*) Moderately sensitive reaction if positive to a dilution of 100 PNU/cc of solution.

RESULTS

Table I shows the number and percentage of allergic individuals with positive skin reactions to 10 PNU/ml and 100 PNU/ml of each pollen extract tested, *i.e.*, the number of individuals with very strong and moderately strong positive skin tests respectively.

TABLE 1.—*Results of skin tests (120 patients).*

Local and scientific names of NIST** pollen extracts	Scratch test	Intradermal test		
	Total no. positive tests	No. positive test 10 PNU /cc	No. positive tests 100 PNU /cc	Total no. positive tests
	Per cent			*Per cent*
1. Yard grass (*Eleusine indica* (L.) Gaertn.)	11 (9.2)	36	41	77 (64.2)
2. Amorseco* (*Antiroppon aeculatus* Retz.)	10 (8.3)	30	42	72 (60.0)
3. Alagasi* (*Dicanthium aristatum* (Poir.) C. E. Hubb.)	24 (20.0)	34	36	70 (58.3)
4. Uray* (*Amaranthus spinosus* Linn.)	2 (1.6)	11	57	68 (56.7)
5. Carabao grass (*Paspalum conjugatum* Berg.)	11 (9.2)	31	36	67 (55.8)
6. Bermuda grass (*Cynodon dactylon* (L.) Pers.)	3 (2.5)	16	48	64 (53.3)
7. Crab grass (*Digitaria* sp.)	0 (0)	21	42	63 (52.5)
8. Para grass (*Brachiaria mutica* (Forssk.) Stapf.)	2 (1.6)	18	40	58 (48.3)
9. Mutha* (*Cyperus rotundus* Linn.)	4 (3.3)	10	38	48 (40.0)
10. Natal grass (*Rynchelytrum repens* (Willd.) C. E. Hubb.)	3 (2.5)	12	34	46 (38.3)
11. Guinea grass (*Panicum maximum* Jacq.)	1 (0.8)	14	27	41 (31.2)
12. Java grass (*Polytrias praemorsa* Hack.)	4 (3.3)	11	25	36 (30.0)
13. Balad batadan (*Sorghum halepense* (L.) Pers.)	12 (10.0)	10	19	34 (28.3)
14. Tridax* (*Tridax procumbens* Linn.)	0 (0)	9	20	29 (24.1)
15. Sunflower** (*Tithonia diversifolia* A. Gray)	1 (0.8)	7	17	24 (20.0)
16. Kogon (*Imperata cylindrica* (L.) Beauv.)	5 (4.2)	11	10	21 (17.5)
17. Makamiya* (*Mimosa pudica* Linn.)	0 (0)	4	16	20 (16.6)
18. Talahib (*Saccharum spontaneum* (L.) subsp. (*indicum*) Hack.)	1 (0.8)	6	13	19 (15.8)
19. Mais (*Zea mays* Linn.)	1 (0.8)	10	9	19 (15.8)
20. Rice (*Oryza sativa* Linn.)	1 (0.8)	4	13	17 (14.1)
21. Foxtail (*Pennisetum polystachyum* (L.) Schultz.)	1 (0.8)	6	10	16 (13.3)
22. Sugar cane (*Saccharum officinarum* Linn.)	2 (1.6)	3	11	14 (11.7)

* A weed.

** National Institute of Science and Technology, Manila.

More than half of the individuals tested or 52.5 per cent and above were found to react positively to six grasses; namely, yard grass, amorsecos, alabang-x, carabao grass, Bermuda grass and crab grass. Yard grass gave the highest percentage of positive skin tests (64 2 per cent). Among the weeds, ural was found to be positive in 56.7 per cent of the test subjects. Other common grasses giving + skin test reactions in 34.2 to 48.3 per cent of the patients were Guinea grass, natal grass, and para grass respectively. Mutha, a weed, was found to give + skin tests in 40 per cent. The rest of the grasses and weeds tested; namely, Java grass, batad-batadan, tridax, sunflower, kogon, makahiya, talahib, mais, rice, foxtail, and sugarcane gave + skin tests in less than a third of the tested individuals. Among these, kogon gave + skin tests in 17.5 per cent while talahib gave + skin tests in 15.8 per cent of the test subjects. Sugarcane gave the lowest number of + skin reactions (11.7 per cent) among the allergic individuals tested.

Scratch tests done on all the 120 test subjects gave + results ranging from 0.8 per cent for Guinea grass, sunflower, talahib, mais, rice, and foxtail to 20 per cent with alabang-x. Negative scratch test results were obtained with extracts of crab grass, tridax, and makahiya.

No untoward reactions, local or systemic, were observed in any of the test subjects on scratch and intradermal testing with the pollen extracts studied.

Negative skin tests were obtained on 18 normal individuals with no allergic personal and family history, using the same pollen extract materials.

Other observations: Blood eosinophilia ranging from 5 to 31 per cent was found in 59 out of 110 allergic individuals (53.6 per cent) with no evidence of parasitic infestations. Nasal eosinophilia occurred in 71 out of 95 test subjects (75.6 per cent) whose nasal smears were examined.

DISCUSSION

It is a well-known and proven fact that pollens constitute one of the principal outdoor inhalant allergens which cause allergic respiratory disease. For a particular pollen to be considered allergenic, aside from its being wind-pollinated, buoyant so that it is easily airborne and produced in large quantities with a widely and abundantly distributed plant source,

it must also be shown to cause allergic disease [Vaughan and Black (1954e)]. The allergic response is produced by the release of vaso-active substances as a result of an enzyme-mediated reaction which is set off by antigen-antibody interaction [Austen and Humphrey (1963)]. In the atopic person, this increased amount of tissue-bound antibody is known as skin-sensitizing antibody or reagin. The skin test is a most convenient immunologic method of showing the presence of specific skin-sensitizing antibodies against a particular allergen. A positive skin reaction, being immunologically specific, is a strong, presumptive evidence of the possible causal allergenic relationship between the symptoms of the afflicted individual and the particular pollen giving the positive test.

In a study of the nitrogen content of extracts from the pollen grains of mais, urai, foxtail millet, Java grass, natal grass, makahiya, and sunflower, Laserna *et al* (1960) mentioned that positive clinical tests were obtained for the first time from their prepared extracts by Rotor.[3] Earlier in 1958, in a preliminary report on the nitrogen content of talahib extract, Laserna *et al* again stated that the latter extract gave positive skin tests when clinically tested by Sevilla and his co-workers,[4] a finding also confirmed by Rotor. However, further details on these clinical observations have remained unpublished. Later in 1966, Vivera made a preliminary report of skin testing results on 34 allergic individuals using NIST-prepared pollen extracts from Bermuda grass, yard grass, talahib, foxtail millet, Java grass, batad-batadan, alabang-x amor-secos, natal grass, kogon, mais, rice, sugarcane, mutha, urai, and tridax. He found that most of these extracts gave positive skin tests except that of natal grass, mais, rice, sugarcane and tridax. The most number of positive reactions were obtained with Bermuda grass and urai weed.

The observations obtained in our study, which was done on a larger group of individuals with allergic respiratory disease, help to answer a long-felt need for more precise and substantial information regarding the allergenic relationship of

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the more common grass and weed pollens in this particular area to the prevalent allergic respiratory diseases by means of skin testing methods. The number of positive skin test results obtained from the 120 test subjects showed that of the 22 most common grass and weed species in the greater Manila area, the most significant in more than half of these allergic individuals are the yard grass, amorsecos, alabang-x, carabao grass, Bermuda grass, crab grass and the urai weed. Of the three most widely abundant grass species reported, yard grass and Bermuda grass gave positive skin results in more than half of the individuals (64.2 per cent and 53.3 per cent, respectively), with yard grass giving the highest number of positive reactions while talahib was found positive in only 19 patients (15.8 per cent). From this finding, though talahib grows abundantly in the surrounding Manila areas, its pollen does not seem to be as strongly antigenic as the yard and Bermuda grasses. Of the five common weeds tested, urai gave the highest number of positive tests (56.7 per cent) and was the only one found to produce skin-sensitizing antibodies in more than half of the persons studied.

All of the six foregoing mentioned grasses and the urai weed grow very densely in uncultivated and waste areas. Bermuda grass is also extensively grown in many gardens. They have all been found to bloom continuously throughout the year except for amorseco which blooms from June to July (Table 2).

Table 2 also shows that most of the grasses and weeds have their heaviest flowering period from the later part of May through December and early January. However, aero-palynological survey had shown that grass pollen is heaviest in the air from October to early January. The greatest amount of pollen in the air, therefore, does not entirely coincide with the time when the grasses or weeds bloom most profusely on the ground. The main reason for this is due to the influence of heavy rainful which usually occurs from late May to October and November. During the rainy season, the strong rains tend to wash out the pollen grains from the opened flowers. In summer, when there is hardly any rain, the grasses are very dry and are rarely in bloom [Payawal and Laserna (1963)].

It has been noted that many individuals with allergic respiratory disease, particularly the asthmatics as in the patients

TABLE 2.—*Flowering months of the grasses and weeds studied* ***

LOCAL AND SCIENTIFIC NAMES	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT.	OCT	NOV.	DEC
1. YARD Eleusine indica (L.) Gaertn	▬	- -	- -	- -	▬	▬	▬	▬	▬	▬	▬	▬
2. AMORSECOS Andropogon aciculatus Retz.						▬	▬					
3. ALABANG-X Dicanthium aristatum (Poir.) C. E. Hubb.	▬	▬	▬	▬	▬	- -	- -	- -	- -	- -	- -	▬
4 URA* Amaranthus spinosus Linn.	▬	- -	- -	- -	- -	- -	▬	▬	▬	▬	▬	▬
5. CARABAO Paspalum conjugatum Berg.	▬	- -	- -	- -	▬	▬	▬	▬	▬	▬	▬	▬
6. BERMUDA Cynodon dactylon (L.) Pers.	▬	- -	- -	- -	▬	▬	▬	▬	▬	▬	▬	▬
7. CRAB Digitaria sp.	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
8. PARA Brachiaria mutica (Forssk.) Stapf.	▬	- -	- -	- -	- -	- -	- -	- -	▬	▬	▬	▬
9. MUTHA* Cyperus rotundus Linn.	- -	- -	- -	- -	- -	▬	▬	▬	- -	- -	- -	- -
10 NATAL Rynchelytrum repens (Willd.) C. E. Hubb	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
11. GUINEA Panicum maximum Jacq	- -	- -	- -	- -	- -	- -	▬	▬	▬	▬		- -
12 JAVA Polytrias praemorsa Hack	▬	▬	- -							▬	▬	▬
13 BATAD BATADAN Sorghum halepense (L.) Pers.	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬
14 TRIDAX* Tridax procumbens Linn	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬
15 SUNFLOWER* Tithonia diversifolia A. Gray	▬	▬	▬	▬	▬					▬	▬	▬
16 KOGON Imperata cylindrica (L.) Beauv.	- -	- -	- -	- -	▬	▬	- -	- -	- -	▬	▬	- -
17 MAKAHIYA* Mimosa pudica Linn	▬	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	▬
18. TALAHIB Saccharum spontaneum (L.) subsp. indicum Hack								▬	▬	▬		
19. MAIS Zea mays Linn	- -	- -	- -	- -	- -	▬	▬	- -	- -	- -	- -	- -
20 RICE Oryza sativa Linn.	▬	▬ **						▬	▬	▬	▬	
21 FOXTAIL Pennisetum polystachyum (L.) Schultz.	- -	- -	- -	- -	- -	- -	- -	- -	- -	▬	▬	▬
22 SUGAR CANE Saccharum officinarum Linn	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -

LEGEND. *A weed
— — — Flowering month
▬ Month of heaviest flowering
**If irrigated

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we have studied, have more attacks during the later part of the year, when the climate is colder. Most of the time, this had been attributed to nonspecific factors which had been observed to precipitate asthmatic symptoms; namely, the change in climate and the increased incidence of respiratory infections during this time of the year. However, now that it has been found that there is increased pollen in the air from October to early January, the latter finding will assume greater importance in the evaluation of the cause of the respiratory symptoms in the allergic individual. A complete allergic work-up of a patient with allergic respiratory disease should, therefore, include testing with the pollens known to be prevalent in his area at the time of occurrence of his symptoms. This study has shown that in the greater Manila area, the more important grasses and weeds to be considered in a larger number of people with allergic respiratory disease are yard grass, amorsecos, alabang-x, carabao grass, Bermuda grass, crab grass and urai weed. However, the other grasses and weeds studied have to be taken into further consideration in a smaller number of allergic persons, especially if the time of occurrence of symptoms coincide with the pollination period of the suspected plant, particularly in the case of grasses with more or less well-defined flowering periods like kogon and talahib or wherever there is intense exposure to cultivated grasses like rice, mais, and sugarcane.

Finally, it must be strongly emphasized that the proper interpretation of a positive skin test in relation to the patient's presenting symptoms always needs close correlation with other factors which can only be obtained from the patient's history and physical findings. The importance of a positive skin reaction can be further confirmed clinically by the improvement of the patient's symptoms on avoidance from exposure or after hyposensitization treatment with the particular pollen antigen.

Observations on immunization studies being done on allergic individuals using the same pollen extracts will be the subject of a future report.

SUMMARY AND CONCLUSION

Skin tests done on 120 individuals with allergic respiratory disease using NIST-produced pollen extracts of 22 grass and weed species found to be most commonly abundant and widely distributed in the greater Manila area showed that yard grass,

amorsecos, alabang-x, Bermuda grass, carabao grass, crab grass, and urai weed gave positive skin reactions in more than half of the test subjects. The highest number of positive skin reactions among the grasses was given by yard grass (64.2 per cent) and urai, among the weeds (56.7 per cent). The importance of these findings in the evaluation of the specific etiology of the allergic individual's respiratory symptoms, especially in correlation with the pollination period of the suspected grass or weed and the patient's history and physical findings, was also discussed.

No untoward reactions, local or systemic, were observed in any of the test individuals, from the use of these extracts.

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FURTHER STUDIES ON THE ALKALOIDS OF VOACANGA GLOBOSA (BLANCO) MERRILL: ISOLATION AND CHARACTERIZATION OF TABERNÆMONTANINE *

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TWO TEXT FIGURES

Previous works on *Voacanga* species have resulted in the isolation and structure determination of several alkaloids. Thomas and Bieman (1968) undertook a detailed investigation of the alkaloids of *Voacanga africana* Stapf, which resulted in the isolation of 19 alkaloids. The isolated alkaloids are enumerated in Table 1.

TABLE 1. *Alkaloids of Voacanga africana Stapf.*

Alkaloid	Structure
Voacamine **	1
Decarbomethoxy-voacamine	2
Voacorine **	3
Vobtusine **	4
Reserpine	5a
Pseudo-Yohimbine	5b
Perakine	6
Iboluteine	7
Voacangine Hydroxyindolenine	8
Voacangine **	9
Ibogamine	10
Coronaridine	11
Ibogaine	12
Voacristine **	13
Iboxygaine	14
Voacangine lactam	15
Vobasine **	16
3 epi α-yohimbine	
β yohimbine	

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* This paper is dedicated to Dr. Alfredo C. Santos on his 70th birthday anniversary, August 15, 1970.

** Previously reported to occur in *V. africana*.

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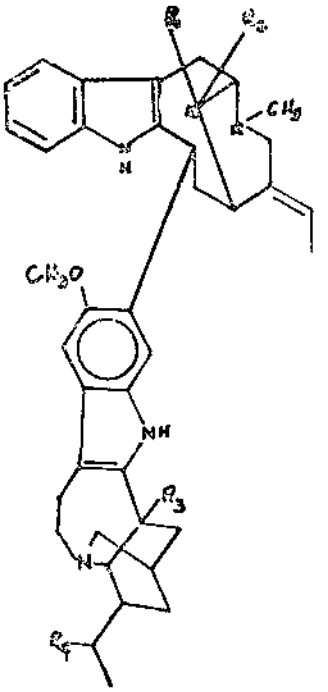
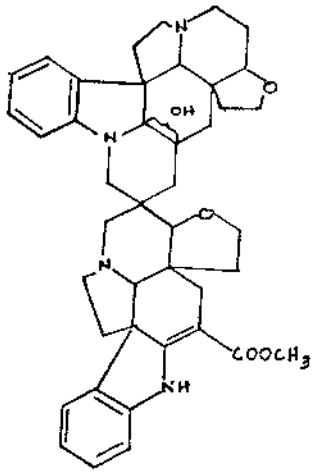
These alkaloids have been classified [Thomas and Biemann (1968)] into six distinct types of indole alkaloids. Of these are the iboga type exemplified by ibogaine (*12*). The second type is the 2-acylindole class of alkaloids to which vobasine (*16*) belong. The dimeric alkaloids might be considered as the third class of alkaloid to which voacamine (*1*) and voacorine (*3*) belong. The dimeric structure (*4*) suggested for vobtusine is not related to any of the skeletal types of alkaloids present in *V. africana*. And thus, this is considered the fourth type of indole alkaloids. The occurrence of perakine (*6*) in *V. africana* accounts for the fifth type of alkaloid that of the ajmaline type. Yohimbine and reserpine (*5a*), having been isolated from *V. africana* make up the sixth class of indole alkaloid.

In the Philippines, there are four native species of *Voacanga* reported in the literature: *Voacanga globosa* (Blanco) Merr. (1950), *V. megacarpa* Quis. and Merr. (1928), *V. delichocalyx* Quis. and Merr. (1928a), and *V. latifolia* Quis. and Merr. (1928b). Owing to great interest in the members of the family Apocynaceæ and the indole alkaloids, an investigation of the stem bark of *V. globosa* (Blanco) Merr. which is the most common and available of the Philippine species of *Voacanga* was initiated. The initial investigation [Lleander (1961)] resulted in the isolation of two crystalline bases which were later [Santos *et al* (1964)] identified as voacamine and vobtusine. In a subsequent report Santos *et al* (1964) report the isolation and identification of these two alkaloids from *V. megacarpa* Merr.



	R_1	R_2	R_3	R_4
Structure *1*	$-COOCH_3$	H	$-COOCH_3$	H
do *2*	$-COOCH_3$	H	H	H
do *3*	$-COOCH_3$	H	$-COOCH_3$	OH



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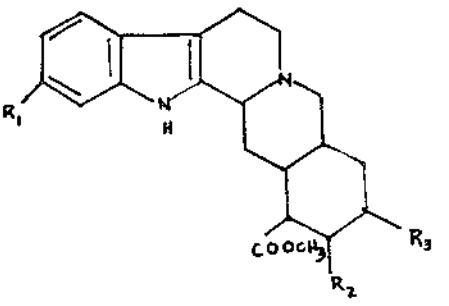
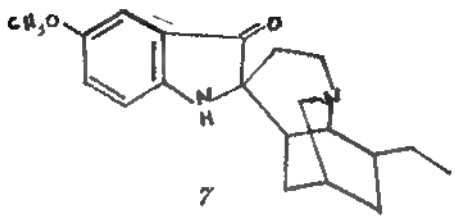


5

	R_1	R_2	R_3
Structure 5a	OCH_3	OCH_3	$OCOC_6H_2(OCH_3)_3$
" 5b	H	OH	H



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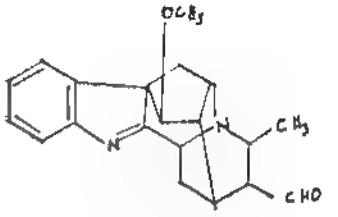

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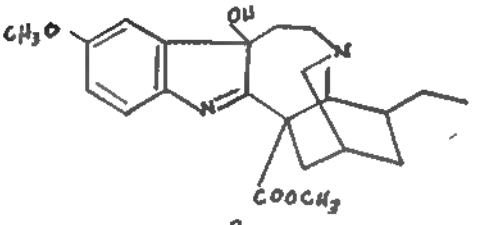

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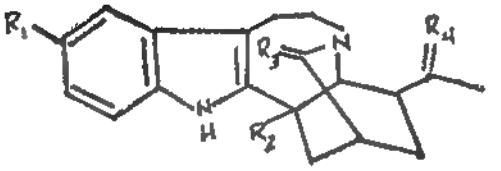
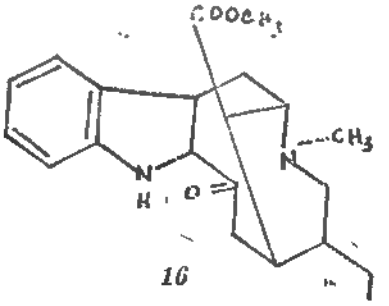


	R_1	R_2	R_3	R_4
Structure *9:*	OCH_3	$COOCH_3$	H	H
do *10:*	H	H	H	H
do *11:*	H	$COOCH_3$	H	H
do *12:*	OCH_3	H	H	H
do *13:*	OCH_3	$COOCH_3$	H	H, OH
do *14:*	OCH_3	H	H	H, OH
do *15:*	OCH_3	$COOCH_3$	O	H



16

Quirin and co-workers (1964) have reported on the isolation from the roots of *Voacanga globosa* (Blanco) Merr. of voacangine, voacamine and alkaloid C. It was reported that alkaloid C is almost identical with vobtusine. The UV and IR spectra are practically superimpossable with those of vobtusine except for the presence of a carbonyl absorption at 1790 cm^{-1} in alkaloid C.

Since preliminary investigations showed that *V. globosa* contained an appreciable amount of indole alkaloids besides those bases which were reported earlier, it was therefore, of interest to reinvestigate *V. globosa*. A methanolic extract of the stem bark of *V. globosa* was placed at our disposal. We are now reporting on the further studies of the alkaloids of *V. globosa* (Blanco) Merr.

Using a different isolation procedure (Scheme 1) from that used in our previous works, we have successfully isolated another crystalline base. The chloroform extract after aqueous extraction was originally intended to be fractionated by gel permeation chromatography. Due to the unavailability of Sephadex LH–20, the reported procedure was used which led to the isolation of the crystalline base.

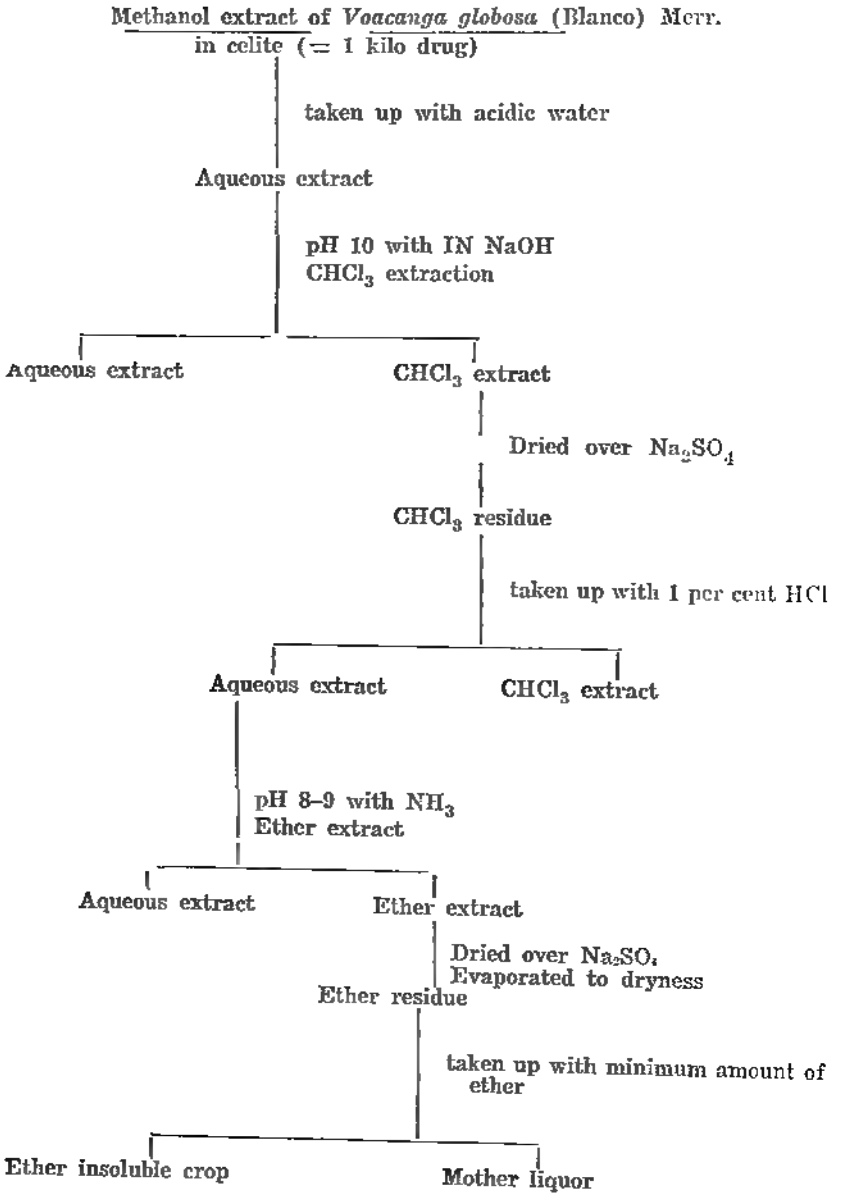
The crystalline base melted at 219 221° (uncorrected). Its UV spectrum[1] (in ethanol) gave maximum absorption at 210 mμ (ε15,400) and 314 mμ (ε11,000) characteristic of a 2-acylindole moiety. The IR spectrum[2] (KBr) showed absorption peaks at 3,300 (NH), 1724 (ester) and 1637 cm^{-1} (2-acylindole). The UV and IR spectra of the isolated alkaloid are in close agreement with those reported[3] for tabernæmontanine ($C_{21}H_{26}O_3N_2$).

In order to elucidate further on the structure of the isolated alkaloid, the nuclear magnetic resonance (NMR) spectrum was recorded.[4] The NMR spectrum showed a three-proton singlet at 2.54 δ which was assigned to a methyl on a nitrogen and another three-proton singlet at 2 61 δ assigned to a methoxyl methyl group. These values are in agreement with those assigned to tabernæmontanine by Cava (1963). Convincing evidence for the close relationship of alkaloid/m.p. 219–221° and

[1, 2] Through the courtesy of Dr. R de Leon, United Laboratories, Inc., Mandaluyong, Rizal.

[3] Physical Data of Indole and Dihydroindole Alkaloids, Eli Lilly.

[4] Through the courtesy of Dr. T. J. Mabry, University of Texas, Austin, Texas. U.S.A. (Trimethylsilane as internal standard).



SCHEME 1. Isolation procedure.

tabernæmontanine to each other was obtained from the mass spectra of the two alkaloids which showed a common fragmentation pattern including the relative peak intensities. The mass spectrum of the crystalline base m.p. 219-221° gave molecular ion peak at m/e 354 which corresponds to $C_{21}H_{20}O_3N_2$.

Figures 1 and 2 show the UV, IR, NMR, and mass spectra of tabernæmontanine.

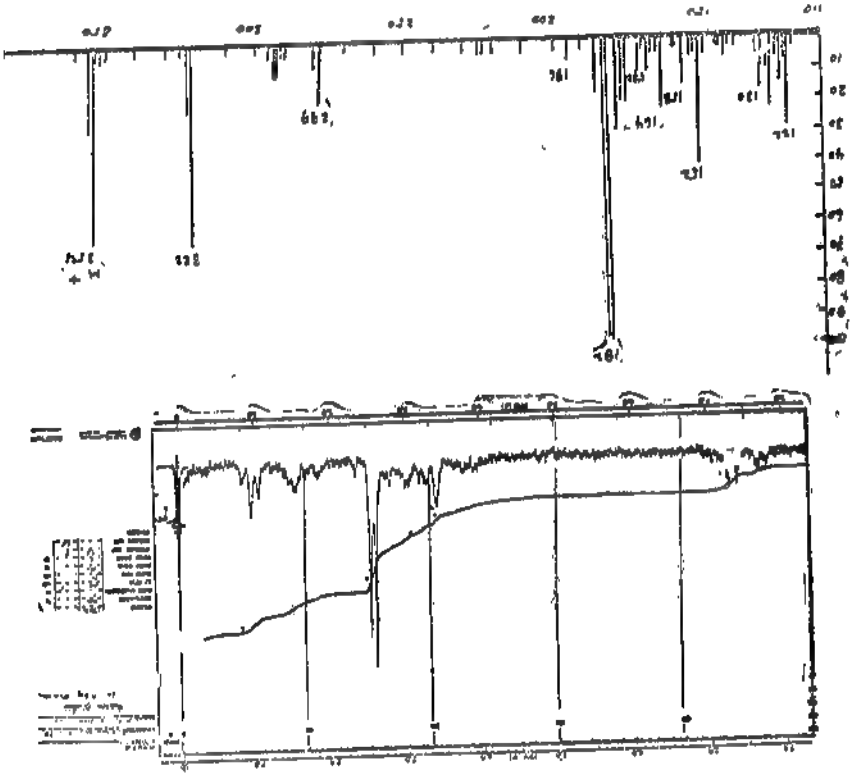
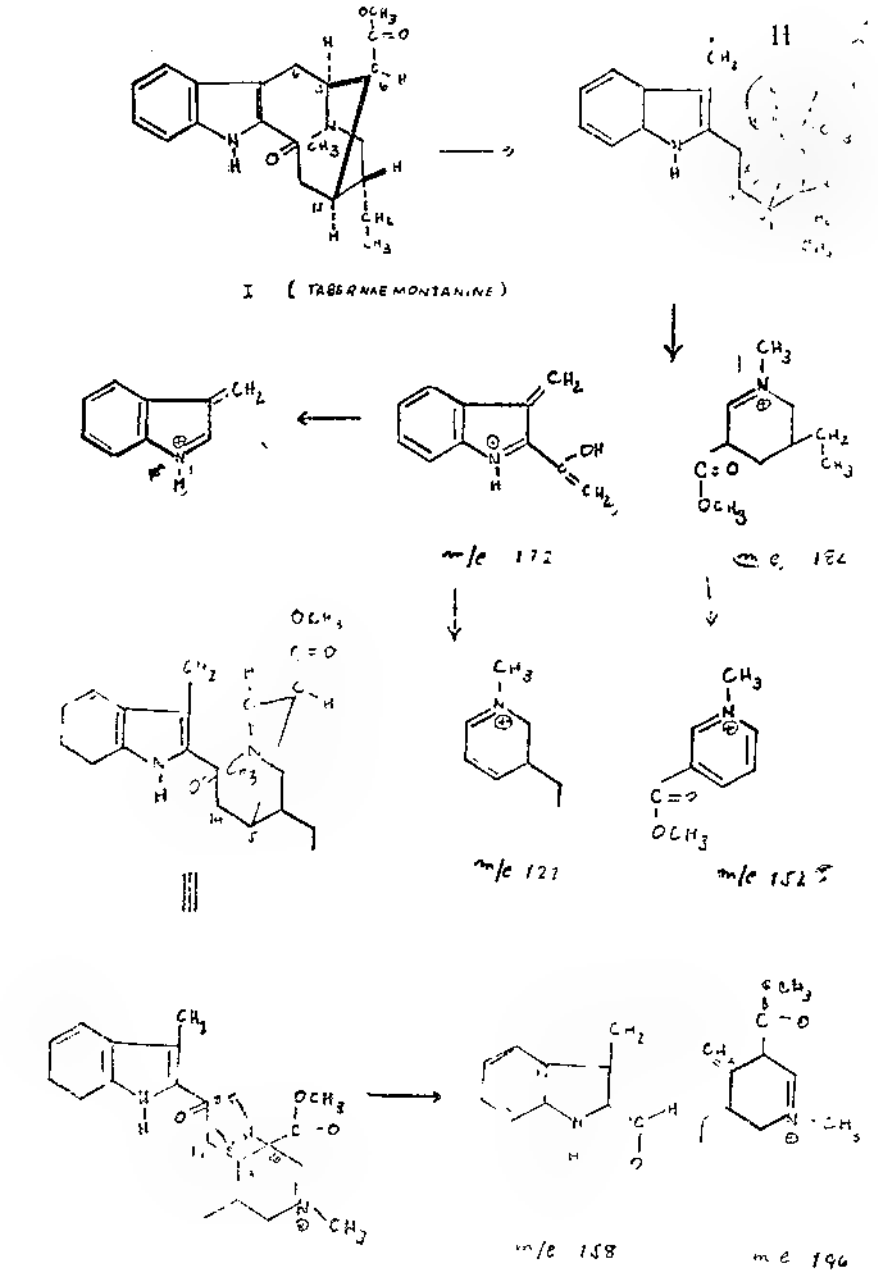


FIG. 1. NMR spectrum of tabernæmontanine (above) and mass spectrum of tabernæmontanine (below).

It is interesting to note that this is the first time that tabernæmontanine has been isolated from *Voacanga*. This fifth alkaloid from *V. globosa* belongs to the 2-acylindole type mentioned earlier.

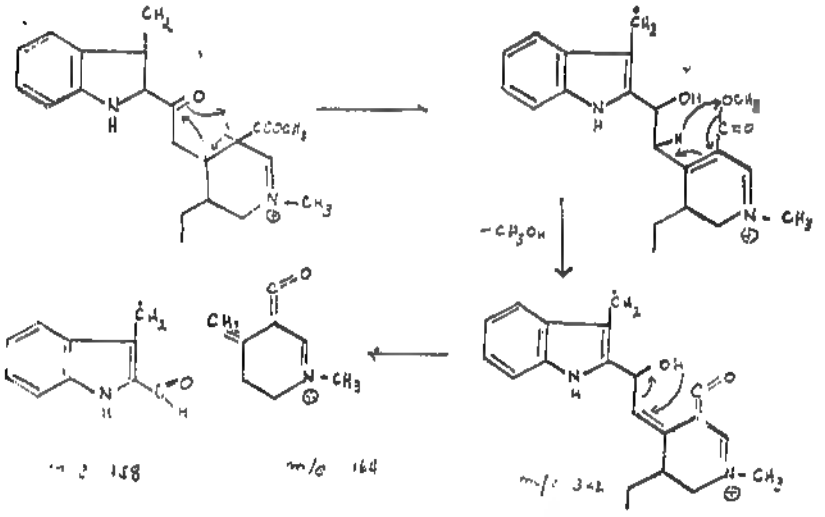
A careful consideration of the mass spectrum of tabernæmontanine [Combes *et al* (1966)] reveals the identification of several fragments. Proposed fragmentation pattern is shown in Scheme II. The principal fragmentation is initiated, accord-


I (TABERNAEMONTANINE)
II
m/e 172
m/e 122
m/e 121
m/e 152
III
m/e 158
m/e 196

Scheme II

ing to the scheme indicated by Budzikiewicz (1963) for vobasine, by rupture of the C_6–C_5 bond. This rupture is then followed by migration of the proton at C_5 to the acyl oxygen and subsequent cleavage of the C_{14}–C_{15} bond, thus, giving rise to ions m/e 172 and m/e 182. Loss of carbomethoxy group from ion m/e 182 yields ion m/e 122. On the other hand, loss of an ethyl chain from ion m/e 182, followed by aromatization leads to ion m/e 152. The presence of ions m/e 158 and m/e 196 may be explained by migration of the C_{16} -proton to C_3 and followed by rupture of C_3–C_{14} bond. Such fragmentation pattern may arise from the well-known β – Cleavage with γ-hydrogen transfer mechanism, since it is a favored fragmentation route of carbonyl with γ-hydrogens.

The presence of ion m/e 322 can only be explained by loss of a molecule of methanol as shown below:



Fragmentation of ion m/e 322 gives m/e 158 and m/e 164 as shown above.

Tabernaemontanine has been in the Cancer Chemotherapy National Service Center program. The screening data[5] indicates that this compound is inactive in (*1*) L–1210 lymphoid

[5] Obtained for us by Dr. J. David Warthen, Jr., Agricultural Research Service, U.S. Dept. of Agriculture Beltsville, Md. from Dr. Harry B. Wood, Jr., National Institutes of Health, Bethesda, Md. U.S.A.

leukemia, (*2*) Walker carcinosarcoma 256 (subcutaneous), and (*3*) Human epidermoid carcinoma of the nasopharynx. The first two, LE and WA, are *in vivo* tumor systems. The third system, 9KB, is an *in vitro* cell culture.

On the other hand, the mother liquor of tabernaemontanine showed activity against leukemia L–1210 and Erlich ascites tumor cells. Further chemical work on the mother liquor is in progress.

ACKNOWLEDGMENT

The authors are grateful to Dr. Rogelio de Leon of the United Laboratories, Inc. Mandaluyong, Rizal for recording the UV and IR and Dr. Tom J. Mabry of the University of Texas, Austin, Texas, U.S.A. for recording the NMR and mass spectra; to the National Institute of Science and Technology, Manila for the facilities put at our disposal. The senior author wishes to acknowledge the financial assistance from the National Research Council of the Philippines.

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QUISUMBING, E., and E. MERRILL (1928b). New Philippine plants. Philip. Jour. Sci. 37: 193.

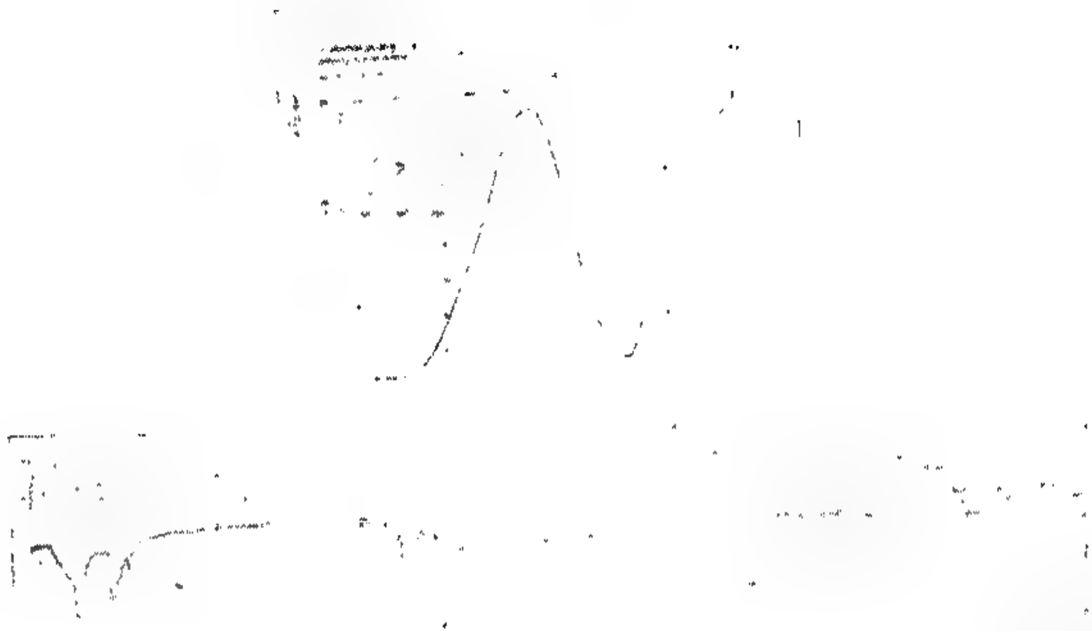

FIG. 2. UV spectrum of tabernæmontanine (above) and IR spectrum of tabernæmontanine (below).

RECLASSIFICATION OF SOME INDO-AUSTRALIAN AND AFRICAN BRACONINÆ AND ROGADINÆ (BRACONIDÆ, HYMENOPTERA)

By CLARE R. BALTAZAR
National Institute of Science and Technology, Manila

The subject of this paper is the reclassification of 145 braconid species, 51 of which were originally described in the genus *Bracon*, 83 in *Iphiaulax*, one or two species in either *Spinaria*, *Myosoma*, *Exothecus* or *Exobracon*, and three rogadine species of *Troporhogas*. These species were described mostly by F. Smith and Cameron, and a few by Bingham, Brues, Strand, and Turner. These types could be found in the British Museum (BM) of Natural History in London or in the Hope Department of Entomology in Oxford University Museum, Oxford. Smith did not indicate where his type specimens were deposited. Cameron, on the other hand, stated in his 1899 paper (Mem. Proc. Manch. Lit. Philos. Soc. 43: 1) that the "species recorded in this and the following papers are now in the collection of Mr. G. A. J. Rothney." Most of the Cameron types from the Rothney collection went to Oxford University. There were instances, however, when specimens labelled by Cameron as types for the same species appear both in London and Oxford. In such cases the specimen in the Oxford Museum was chosen as the true type or lectotype.

Subfamily BRACONINAE

In the past many species of Braconinæ were described either in *Bracon* or *Iphiaulax*. Present-day grouping would place these species in different genera in the subfamily Braconinæ.

The first three genera discussed, namely, *Bracon*, *Campyloneurus* and *Pachybracon* have the following characteristics in common: Tergite 1 shorter than or at most as long as its apical width; tergite 2 transverse or 0.4 to 0.5 as long as its apical width; nervulus usually forming a straight line with basal vein, the latter forming a 75° to 80° angle with subcosta; head usually transverse from dorsal view; species mostly small or medium-sized.

Genus BRACON Fabricius

Bracon FABRICIUS (1804). Systema Piezatorum, p. 102.
Type: *Ichneumon minutator* Fabricius. Designated by Intl. Comm. Zool. Nomencl. Op. 162, 1945.
Synonyms: *Braco* Wesmael, *Microbracon* Ashmead, *Habrobracon* Johnson, *Macrodyctium* Ashmead, *Tropidobracon* Ashmead.
Distribution: Worldwide.

The species listed below have the following characteristics: abscissa 1 of cubitus straight; cubital cell 2 equal to or shorter than cubital cell 3; tergite 3 to 5 usually without a transverse groove apically; recurrent vein antefurcal or interstitial.

BRACON CLANES (Cameron), comb. nov.

Iphiaulax clanes CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 151. Type: ♀, Dunbrody, Cape Colony (BM 3c, 378).

BRACON DISTINCTISULCATUS (Strand), comb. nov.

Iphiaulax distinctisulcatus STRAND (1912). Arch. Naturg. Jahrg. 78A (6): 51, 63. Type: ♀, Siluas, Sambas, W. Borneo (BM 3c 413).

Six species of *Bracon* were also examined and believed to belong in the genus *Bracon:*

Bracon australasicus CAMERON (1912). Proc. Linn. Soc. N. S. Wales 37: 193. Type: ♀, N. S. Wales (BM 436).
Bracon basalis SMITH (1858). J. Proc. Linn. Soc. Zool. 3: 171. Type: ♀, Aru (Oxford).
Bracon firmus CAMERON (1900). Mem. Proc. Manch. Lit. Philos. Soc. 44: 84. Type: ♂, Khasia Hills, India (Oxford, tip of abdomen damaged).
Bracon nitidus SMITH (1858). J. Proc. Linn. Soc. Zool. 3: 175. Type: ♀, Aru (Oxford).
Bracon pilitarsis CAMERON (1912). Proc. Linn. Soc. N. S. Wales 37: 193. Type: ♀, N. S. Wales (BM 435).
Bracon umbratilis CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 74. Type: ♀, Khasia Hills, India (Oxford).—DOVER (1925). Ent. Mitt. 14: 39. (comb. nov.) *Campyloneurus.*

Genus CAMPYLONEURUS Szepligeti

Campyloneurus SZEPLIGETI (1900). Term. Fuzet. 23: 51.
Type: (*Campyloneurus bicolor* Szepligeti)=*Campyloneurus bicolorimus* Viereck. Designated by Viereck (1911).
Distribution: Indo-Australian and African.

This genus may be differentiated from *Bracon* in having the abscissa 1 of cubitus curved at base, cubital cell 2 as long as cubital cell 3, tergites 3 to 5 each with a transverse groove along its apical margin, and recurrent vein usually interstitial. The species listed below are transferred in the genus *Campyloneurus.*

CAMPYLONEURUS ABDOMINALIS (Smith), comb nov.
Bracon nigripennis SMITH (1858). J. Proc. Linn. Soc. Zool. 3: 175. Type: ♀, Aru (Oxford).

CAMPYLONEURUS BRUNNEO-MACULATUS (Cameron), comb. nov.
Iphiaulax brunneo-maculatus CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 119. Type: ♀, Kuching, Borneo (BM 400).

CAMPYLONEURUS CAMPBELLI (Cameron).
Iphiaulax campbelli CAMERON (1907) Ann. Mag. Nat. Hist. (7) 19: 175. Type: ♀, Sikkim, India (BM 408).—DOVER (1925). Ent. Mitt. 14: 29. (comb. nov.) *Campyloneurus.*

CAMPYLONEURUS CILLES (Cameron), comb. nov.
Iphiaulax cilles CAMERON (1905). J. Str. Brit. Roy As. Soc. 42: 32. Type: ♀, Kuching, Borneo (BM 404).

CAMPYLONEURUS CRASSIPES (Smith), comb. nov.
Bracon crassipes SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 196. Type: ♀, Singapore (Oxford).

CAMPYLONEURUS CRASSITARSIS (Cameron), comb nov
Iphiaulax crassitarsis CAMERON (1903). J. Str. Brit. Roy As. Soc. 39: 112. Type: ♀, Kuching, Borneo (BM 392).

CAMPYLONEURUS DECLARATUS (Cameron), comb. nov.
Bracon declaratus CAMERON (1899). Mem. Proc. Lit. Philos. Soc. 43: 79. Type: ♀, Khasia Hills, India (Oxford).

CAMPYLONEURUS EXOLETUS (Smith), comb. nov.
Bracon exoletus SMITH (1858). J. Proc. Linn. Soc. Zool. 3: 175. Types: 2 ♀ ♀, Aru (Oxford, 1 ♀ with abdomen missing); Lectotype: ♀ with abdomen intact, Aru (Oxford).

CAMPYLONEURUS HARAGAMENSIS (Cameron), comb nov
Iphiaulax haragamensis CAMERON (1905). Spolia Zeyl. 3: 86. Type: ♀, Haragam, Ceylon (BM 401).

CAMPYLONEURUS HIRPINUS (Cameron), comb. nov.
Iphiaulax hirpinus CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 115. Type: ♀, Kuching, Borneo (BM 395).

CAMPYLONEURUS KIRBYI (Cameron), comb nov.
Iphiaulax kirbyi CAMERON (1905). Spolia Zeyl. 3. 85. Types: 2 ♀ ♀, Kandy, Ceylon (BM 403); Lectotype: ♀, Kandy, Ceylon with data "9-02, Cameron coll. 1909-182." (BM 403).

CAMPYLONEURUS SAITIS (Cameron), comb nov.
Iphiaulax saitis CAMERON (1909). Soc. Ent. 24: 138. Types: 1 ♂, 1 ♀, Kuching, Borneo; Lectotype: ♀, Kuching, Borneo (BM 402).

CAMPYLONEURUS SIKKIMENSIS (Cameron), comb. nov.
Iphiaulax sikkimensis CAMERON (1907). Ann. Mag. Nat. Hist. (7) 19: 174. Type: ♀, Sikkim, India (BM 406).

CAMPYLONEURUS TRIMACULATA (Cameron), comb. nov.
Spinaria trimaculata CAMERON (1900). Mem. Proc. Manch. Lit. Philos. Soc. 44: 81. Type: ♀, Khasia Hills, India (Oxford). —WATANABE (1937). Ins. Mats. 11 (3): 115. (listed.)

Genus PACHYBRACON Cameron

Pachybracon CAMERON (1908). Ent. 41: 295.
Type: *Pachybracon fortipes* Cameron. By monotypy.
Distribution: Oriental.

This genus is similar to *Camploneurus* and *Bracon* in the oval shape of the gaster, the smooth and shiny thorax and propodeum, and the nervulus forming a straight line with the basal vein, the latter forming a 75° to 80° angle with the subcosta. However, the female of *Pachybracon* is different from the two genera in that the ovipositor is thickened and the ovipositor tip is blunt. The ovipositor is about ½ as long as the fore wing.

A female cotype of *Pachybracon fortipes* was examined in the British Museum and it has the eyes pubescent (no species of *Bracon* and *Campyloneurus* from the Philippines has the eyes hairy); the notaulus is deep; no scutellar fovea is present; the hind femur, tibia and tarsus are bristly but less hairy in the tibia and tarsus of middle leg; the basal ½ of wings is brown, the distal ½ is opaque white.

PACHYBRACON CARNASIUS (Cameron), comb nov
Iphiaulax carnasius CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 119. Type: ♀, Kuching, Borneo (BM 39).

Genus MYOSOMA Brulle

Myosoma BRULLE (1846). Hist Nat. Ins. Hym. 6: 450.
Type: *Myosoma hirtipes* Brulle. Designated by Viereck (1911).
Synonyms: *Acanthobracon* Kriechbaumer, *?Acanthobracon* Szepligeti, *?Trichodoryctes* Szepligeti.
Distribution: Indo-Australian and Neotropical.

This genus may be recognized from other genera by the flat and long 1st tergite which is about 3 times as long as its apical width and with a wide membrane laterally. The tergites are all smooth and shiny. Like *Bracon*, *Campyloneurus*, and *Pachybracon*, tergite 2 is transverse, the nervulus forms a straight line with the basal vein, the latter forming a 75° to 80° angle with subcosta.

MYOSOMA FEROX (Smith), comb nov.
Bracon ferox SMITH (1864). J. Linn. Soc. Zool. 8: 66.
Type: ♀, New Guinea. Neotype: ♀, Makassar, Celebes, with a handwritten label "*Bracon ferox* Smith" (Oxford).

Genus MACROBRACON Szepligeti

Macrobracon SZEPLIGETI (1902). Term. Füzet. 25: 44.
Type: *Macrobracon concolor* Szepligeti. Designated by Viereck (1914).
Distribution: Indo-Australian.

The species in this genus have bifid claws; tergites 2 to 4 have a hump on each apical corner and a pimplelike elevation midbasally; ovipositor is short, not longer than ½ the length of the fore wing. These are large species with thickset abdomen.

MACROBRACON FULVOPILOSUS (Cameron).
Iphiaulax fulvopilosus CAMERON (1905). Spolia Zeyl. 3: 83.
Type: ♀, Kandy, Ceylon (BM 336). The ♀ specimen in Oxford Museum with a handwritten label "*Iphiaulax fulvopilosus* Cameron" from Makassar, Celebes, is not the type—TURNER (1918). Trans. Ent. Soc. London, p. 97. (comb. nov.) *Macrobracon*.

MACROBRACON GRAVIDUS (Smith), comb. nov.
Bracon gravidus SMITH (1861). J. Linn. Soc. Zool. 8: 66
Type: ♀, New Guinea. Neotype: ♀, Makassar, Celebes, with a handwritten label "*Bracon gravidus* Smith" (Oxford).

MACROBRACON NIGRIPENNIS (Smith), comb. nov.
Bracon nigripennis SMITH (1858). J. Proc. Linn. Soc. Zool. 3: 177.
Type ♀, Aru (Oxford).

Genus CHAOILTA Cameron

Chaolta CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 80.
Type: *Chaolta lanciluta* Cameron. By monotypy.
Synonyms: *Odontoscapus* Kriechbaumer, *Blastomorpha* Szepligeti, *Platybracon* Szepligeti.
Distribution: Indo-Australian.

The genus is easily recognized because the thorax and abdomen are flat and depressed and the pronotum is prolonged into a neck, the scape is excised basally; in the female the face has usually a protrusion below the antennal sockets.

CHAOILTA AMESTRIS (Cameron), comb. nov.
Iphiaulax amestris CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 115. Type: ♀, Kuching, Borneo (BM 385).

CHAOILTA HIMALAYENSIS (Cameron), comb. nov.
Bracon himalayensis CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 70. Type: ♀, Khasia Hills, India (Oxford, abdomen missing).

CHAOILTA INSULARIS (Cameron), comb. nov.
Platybracon insularis CAMERON (1911). Proc. Linn. Soc. N. S. Wales 36: 308. Type: ♀, Solomon Is. (BM C10). Identification label was inadvertently interchanged with *Platybracon nigriceps*.

CHAOILTA NIGRICEPS (Cameron), comb. nov.

Platybracon nigriceps CAMERON (1911). Proc. Linn. Soc. N. S. Wales 36: 338. Type: ♀, Gin Gin, Queensland (BM 609). Identification label was inadvertently interchanged with *Platybracon insularis*.

CHAOILTA VULTUOSUS (Smith), comb. nov.

Bracon vultuosus SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 125. Type: ♀, Singapore (Oxford).

Types of "*Chaolta*" species that were examined and believed to belong in *Chaoilta* are:

Chaolta fuscipennis CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 120. Type: ♀, Kuching, Borneo (BM 593).

—*Chaolta ruficeps* CAMERON (1905). J. Str. Brit. Roy. Soc. 44 101. Type: Buatal, Borneo (BM 595). *New synonymy.*

Chaolta lutea CAMERON (1906). J. Str. Brit. Roy. As. Soc. 44: 102. Type: ♀, Kuching, Borneo (BM 594).

Chaolta maculifrons CAMERON (1905). J. Str. Brit. Roy. As Soc 42: 50. Type: ♀, Kuching, Borneo (BM 597).

Chaolta sulcata CAMERON (1905). J. Str. Brit. Roy. As. Soc. 42: 50 Type: ♀, Kuching, Borneo (BM 596).

Chaolta trituberculata CAMERON (1905). J. Str. Brit. Roy. As. Soc Type: ♀, Kuching, Borneo (BM 414).

Genus ATANYCOLUS Foerster

Atanycolus FOERSTER (1862). Verh. naturh. Ver. preuss. Rheinlande 19: 238. Type: *Ichneumon denigrator* Linnaeus. By monotypy and original designation.

Synonyms: *Coelobracon* Thomson, *Melanobracon* Ashmead, *Atanycolidea* Viereck.

Distribution: Worldwide.

The genus has the base of the scape excised as in *Chaolta*, however, the thorax, propodeum and tergites are not depressed and the notauli are deeply impressed.

ATANYCOLUS EXCERPTA (Turner), comb. nov.

Medinoschiza excerpta TURNER (1920). Ann. Mag. Nat. Hist. (9) 5: 92. Type: ♀, Tonkin, Indo-China (BM 568).

ATANYCOLUS FUSCIPENNIS (Cameron), comb. nov.

Myosoma fuscipennis CAMERON (1902). J. Str. Brit. Roy. As. Soc. 37: 40. Type: ♀, Borneo (BM 544).

ATANYCOLUS TRICHIURA (Cameron), comb. nov.

Myosoma trichiura CAMERON (1902). J. Str. Brit. Roy. As. Soc. 37: 39. Type: ♀, Sarawak, Borneo (BM 543).

The nine genera that follow have the following characteristics in common: Tergite 1 longer than its apical width, from 1.5 to 3 times as long as apical width; nervulus not forming

a straight line with basal vein, the basal vein slanting or oblique and forming a 45° to 60° angle with subcosta; head usually cubical; species mostly large to medium-sized.

Genus ISCHNOBRACON Baltazar

Ischnobracon BALTAZAR (1968). Pacific Insects 5: 587.
Type: *Ischnobracon bakeri* Baltazar. By original designation.
Distribution: Oriental (Borneo, India, Philippines).

The genus is readily recognized by the shiny and impunctate triangular area at the base of tergites 2 to 4; tergite 2 is 1.2 to 1.5 times its apical width; the notauli are deeply impressed and extend to apical margin of mesoscutum; the subgenital plate in the ♀ is triangular in side view and does not extend beyond tip of last tergite; the ovipositor sheath is about ½ as long as fore wing. A more detailed description of the genus is given in the publication cited above.

The following species possess the above characteristics and are now transferred in *Ischnobracon*.

ISCHNOBRACON INDISCRETUS (Cameron), comb. nov.
Bracon indiscretus CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 71. Type: ♀, Khasia Hills, India (Oxford).

ISCHNOBRACON LABORIOSUS (Smith), comb. nov.
Bracon laboriosus SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 126. Type: ♀, Sarawak, Borneo (Oxford).

ISCHNOBRACON V-MACULA (Cameron), comb. nov.
Bracon v-macula CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 62. Types: 2 ♀, Khasia Hills, India (Oxford and BM 437); Lectotype: ♀, Khasia Hills, India (Oxford); Paralectotype: ♀, Khasia Hills, India (BM 437).

Bracon orientalis CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 63. Types: 2 ♀, Khasia Hills, India (Oxford and BM 432); Lectotype: ♀, Khasia Hills, India (Oxford); Paralectotype: ♀, Khasia Hills, India (BM 432, abdomen missing). *New synonymy.*

The two females in Oxford, each bearing a handwritten label of *Bracon v-macula* and *orientalis*, are so similar to each other and the only difference is the entirely fulvous abdomen and dark streak on the ventral side of hind femur of *v-macula*, whereas in *orientalis* tergites 4 to 7 are darkish (but probably due to deterioration) and the hind femur is entirely fulvous

(however, the specimen of *orientalis* in London has a small ventral dark streak on hind femur).

All the other types of species described by Cameron in 1899 are found in Oxford, hence the preference for the Oxford specimen as lectotype.

Genus GRONAULAX Cameron

Gronaulax CAMERON (1910). Soc. Ent. 25 (6): 23.
Type: *Gronaulax piloselius* Cameron. By monotypy.
Synonym: *Neuraulax* Roman.
Distribution: Oriental (Borneo and Philippines).

In this genus the basal triangular area on the second tergite is wrinkled, tergite 2 is 1.2 to 1.5 times its apical width and there are 2 apically convergent lateral carinæ; the ♀ subgenital plate is apically elongate and extends beyond the tip of the last tergite; the ovipositor sheath is about 2 times the length of the fore wing; the notauli are usually deeply impressed.

ISCHNOBRACON LABORIOSUS (Smith), comb. nov.
Iphiaulax leptogaster CAMERON (1905). J. Str. Brit. Roy. As. Soc. 42: 47. Type: ♂, Kuching, Borneo (BM 387).
Iphiaulax octoforcatus CAMERON (1907). J. Str. Brit. Roy. As. Soc. 48: 4. Type: ♂, Kuching, Borneo (BM 396). *New synonymy.*

The six genera that follow have the 2nd tergite as long as or shorter than its apical width (excepting ♂ ♂ of *Euurobracon*). All have the ovipositor long with the exception of *Hybogaster*. Three genera, namely, *Euurobracon*, *Bathyaulax* and *Hybogaster* have no triangular area on the 2nd tergite and the scape is short, ranging from 1 to 1.5 times as long as its diameter. In contrast to the last three genera discussed in this paper, namely, *Cratobracon*, *Sigalphogaster* and *Iphiaulax*, there is a midbasal triangular area on the 2nd tergite; the scape is long, from 2 to 4 times as long as its diameter except in *Iphiaulax* where the scape is 1 to 1.5 times as long as its diameter.

Genus EUUROBRACON Ashmead

Euurobracon ASHMEAD (1900). Proc. U. S. Natl. Mus. 23: 45.
Type: (*Bracon penetrator* Smith) = *Euurobracon yokohamae* (Dalla Torre). By monotypy.
Synonyms: *Delmira* Cameron, *Exobracon* Szepligeti, *Lissobracon* Cameron.
Distribution: Palearctic (Japan, Korea) and Indo-Australian; ? African.

In this genus the recurrent vein is strongly antefurcal, its distance from intercubitus 1 is ½ or equal to the length of

abscissa 1 of radius; the nervulus is postfurcal. The face is wide. The 1st tergite has a deep median groove on the basal 1/3. The ovipositor sheath is about 1.5 times as long as the fore wing or longer.

EUROBRACON CEPHALOTES (Smith), comb. nov.

Bracon cephalotes SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 123. Type: ♀, Sarawak, Borneo (Oxford).

Delmira triplagiata CAMERON (1900). Mem. Proc. Manch. Lit. Philos. Soc. 44: 88. Type: ♀, Khasia Hills, India (Oxford). *New synonymy.*

EUROBRACON QUADRICEPS (Smith).

Bracon quadriceps SMITH (1860) (Nec 1857). J. Proc. Linn. Soc. Zool. 4: 141. Types: ♀ ♀, Batchian, Weigen, ?Eldos; Lectotype: ♀, with a handwritten label *"Bachian"* and *"Bracon quadriceps* Sm." (Oxford).—SZEPLIGETI (1904). Gen. Insect. fasc. 22a: 47. (comb. nov., syn.) *Exobracon.*—ROMAN (1913). Arkiv. Zool. 8 (15): 45. (comb. nov.) *Eurobracon.*

Bracon impossibilis DALLA TORRE (1898). Cat. Hym. 4: 273. Type: ♀, Batchian.

Genus BATHYAULAX Szepligeti

Bathyaulax SZEPLIGETI (1906). Ann. Mus. Nat. Hung. 4: 550, 559. Type: *Bathyaulax cyanogaster* Szepligeti. Designated by Viereck, 1914.

Distribution: Africa, Asia.

The female is readily recognized by the long ovipositor that has an augerlike tip, with three or four constrictions at apex. The 1st tergite has no midlongitudinal groove basally; the 2nd tergite has no median triangular area near base; the 3rd tergite has a triangular area marked off on its basal corner. The recurrent vein is interstitial or slightly anterfurcal; the nervulus is postfurcal.

BATHYAULAX PLUMOSUS (Kirby).

Bracon plumosus KIRBY (1896). Ann. Mag. Nat. Hist. (6) 18: 262. Type: ♀, Ogove, Africa (BM 431).—TURNER (1917). Ann. Mus. Nat. Hist. (8) 20: 242. (comb. nov.) *Bathyaulax.*

BATHYAULAX STANLEYI (Cameron), comb. nov.

Iphiaulax stanleyi CAMERON (1912). Ann. Soc. Ent. Belg. 56: 368. Type: ♀, Leopoldville, Belgian Congo (Congo Mus.). The ♀ specimen in the British Museum which was labelled as this species and given a type No. 374 bears no locality label.

BATHYAULAX STRENUUS (Cameron), comb. nov.

Iphiaulax strenuus CAMERON (1909). Arch. Mat. Naturv. Krist. 30 (10): 6, 14. Type: ♀, Delagoa Bay (Berlin Mus.). The ♀ specimen in the British Museum which was labelled as this species

and given a type No. 375 bears the same type locality label CAMERON (1904). Rec. Albany Mus. Grahamstown S. Africa 1: 1 5. (comb. nov.) *Iphiaulax*.

Bracon bicolor BRULLE (1846). Hist. Nat. Ins. Hym. 4: 112. Type: ♀, Africa.—BRUES (1924). Ann. S. Afr. Mus. 19. 61. (syn.) *Iphiaulax*.

Three species of *Iphiaulax* described by Cameron from male specimens, namely, *rubrinervis*, *spilonotus* and *whitei*, seem to belong in *Bathyaulax* but because of insufficient knowledge about the characteristics of the male of *Bathyaulax*, they are retained in *Iphiaulax*.

Genus HYBOGASTER Szepligeti

Hybogaster SZEPLIGETI (1906). Ann. Mus. Nat. Hung. 4: 601. Type: *Hybogaster gibberosus* Szepligeti. By monotypy. Distribution: Indo-Australian.

The female of this genus has the ovipositor short, thickened and curved downwards; the length of ovipositor does not exceed the length of entire abdominal tergites. The nervulus is interstitial and the recurrent vein is interstitial or slightly antefurcal. The 3rd tergite has a triangular area marked off on its basal corner.

It differs from *Iphiaulax* in that the 2nd tergite has no triangular area midbasally and its ovipositor is short.

HYBOGASTER ACRAGAS (Cameron), comb. nov.

Iphiaulax acragas CAMERON (1903). J. Sta. Brit. Roy. As. Soc. 37: 33. Type: ♀, Borneo (BM 316).

HYBOGASTER HAUNDRAWENSIS (Cameron), comb nov.

Iphiaulax haundrawensis CAMERON (1907). Ann. Mag. Nat. Hist. (7) 19: 171. Type: ♀, Haundraw Valley, Tenasserim, India (BM 339).

HYBOGASTER JEJUNUS (Cameron), comb. nov.

Bracon jejunus CAMERON (1899). Mem. Proc Manch. Lit. Philos. Soc. 43: 78. Type: ♀, Khasia Hills, India (Oxford).

HYBOGASTER MALAYANUS (Cameron), comb. nov.

Iphiaulax malayanus CAMERON (1901). Proc. Zool Soc. London 2: 43. Type: ♀, Singora, Malay Peninsula (BM 349).

HYBOGASTER XANTHOPSIS (Cameron), comb. nov.

Iphiaulax xanthopsis CAMERON (1905). Spolia Zeyl. 3: 82. Type: ♀, Elephant Pass, Ceylon.—DOVER (1925). Ent. Mitt. 14: 40. (syn.).

Iphiaulax spilocephalus CAMERON (1907). J. Nat. Hist. Soc Bombay 17: 584. Types: ♂, ♀, Deesa, India; Lectotype: ♀, Deesa, India (BM 353).

HYBOGASTER VARIPALPIS (Cameron), comb. nov.
Iphiaulax varipalpis CAMERON (1906). Ann. S. Afr. Mus. 5: 48. Type: "♂"=♀, Transvaal, Cape Colony (BM 350).—KNIGHT (1930). East Afr. J. 5: 65.—CROWE (1962). Ent. Soc. South Afr. J. 25: 369.

HYBOGASTER VARIPENNIS (Cameron), comb. nov.
Iphiaulax varipennis CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 110. Type: ♀, Matang, Borneo (BM 361).

Genus CRATOBRACON Cameron

Cratobracon CAMERON (1901). Proc. Zool. Soc. London 1: 226. Type: *Cratobracon ruficeps* Cameron. By monotypy.
Distribution: Indo-Australian.

Cratobracon is similar to *Sigalphogastra* in that the 2nd tergite has a pair of carinæ that converge apically and the segment bears a small tooth on each apical corner. It differs from *Sigalphogastra*, however, in having a long scape, length about 3 to 4 times its diameter, and the presence of a raised central area and a midlongitudinal carina on the 1st tergite.

The type of the genus, *Cratobracon ruficeps* Cameron (BM Type No. 156) has the apical margin of the clypeus turned upward, the notauli are deep and 2nd tergite has a midlongitudinal carina in addition to the two oblique carinæ; tergites 1 to 4 are wrinkled and longitudinally striate, the rest are impunctate.

CRATOBRACON JACULATUS (Smith), comb. nov.
Bracon jaculatus SMITH (1860). J. Proc. Linn. Soc. Zool. (Suppl.) 4: 141. Type: ♀, Batchian. Neotype: ♀, Makassar, Celebes, with a handwritten label "*Bracon jaculatus* Sm." (Oxford).

CRATOBRACON RETICULATUS (Cameron), comb. nov.
Iphiaulax reticulatus CAMERON (1905). J. Str. Brit. Roy. As. Soc. 42: 39. Type: ♂, Mt. Matang, Borneo (BM 376).

Genus SIGALPHOGASTRA Cameron

Sigalphogastra CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 124. Type: *Sigalphogastra ashmeadi* Cameron. By monotypy.
Distribution: African and Indo-Australian.

It differs from *Cratobracon* in that the scape is shorter, length only about 2 times its diameter. There is no midlongitudinal carina on the 1st tergite.

SIGALPHOGASTRA AETHIOPICA (Cameron), comb. nov.
Iphiaulax aethiopicus CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 153. Type: ♀, Dunbrody, Cape Colony (BM 377).

Iphiaulax melanosoma (Brulle), *teste* BRUES (1926). Proc. Amer. Acad. Arts Sci. 61 (8): 221.
Merinotus striatus Szepligeti, *teste* BRUES (1921). Ann. S. Afr. Mus. 19: 61.

SIGALPHOGASTRA CAPENSIS (Cameron), comb. nov.
Iphiaulax capensis CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 149. Type: ♀, Dunbrody, Cape Colony (BM 379).—FAHRINGER (1926). Opusc. Brac. 1 (2-3): 167. (comb. nov.) *Merinotus.*

SIGALPHOGASTRA COMBUSTUS (Smith), comb. nov.
Bracon combustus SMITH (1860). J. Proc. Linn. Soc. Zool. (Suppl.) 4: 65. Type: ♀, Makassar, Celebes (Oxford). SZEPLIGETI (1901). Termes. Fuzetek. 24: 367. (comb. nov.) *Iphiaulax.*—SZEPLIGETI (1906). Ann. Mus. Natl. Hung. 4: 565. (comb. nov.) *Merinotus.*

SIGALPHOGASTRA ERNESTI (Cameron).
Iphiaulax ernesti CAMERON (1905). Spolia Zeyl. 3: 84.
Type: ♀, Peradeniya, Ceylon (BM 390).—DOVER (1925). Ent. Mitt. 14: 39. (comb. nov.) *Sigalphogastra.*

SIGALPHOGASTRA FOVEATUS (Smith), comb. nov.
Bracon foveatus SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 126. Types: ♀ ♀, Borneo and Malacca; Lectotype: ♀, Singapore (Oxford).

SIGALPHOGASTRA GREENI (Cameron).
Iphiaulax greeni CAMERON (1905). Spolia Zeyl. 3: 83.
Types: 2 ♀ ♀, Peradeniya, Ceylon (London); Lectotype: ♀, Peradeniya, Ceylon (BM 388).—DOVER (1925). Ent. Mitt. 14: 39 (comb. nov.) *Sigalphogastra.*

SIGALPHOGASTRA HAVILANDI (Cameron), comb. nov.
Iphiaulax havilandi CAMERON (1906). Ann. S. Afr. Mus. 5: 42. Type: ♀, Natal, Cape Colony (S. African Mus.). The ♀ specimen tagged as BM Type No. 405 is not the type. It bears a locality label "Cape" and a handwritten label "*Iphiaulax havilandi* Cam."

SIGALPHOGASTRA KUCHINGENSIS (Cameron), comb. nov.
Iphiaulax kuchingensis CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 104. Type: ♀, Kuching, Borneo (BM 382).

SIGALPHOGASTRA ORNATICORNIS (Cameron), comb. nov.
Iphiaulax ornaticornis CAMERON (1905). J. Str. Brit. Roy. As. Soc. 42: 48. Type: ♀, Kuching, Borneo (BM 378).

SIGALPHOGASTRA PALLIDIFRONS (Smith), comb. nov.
Bracon pallidifrons SMITH (1858). J. Proc. Linn. Soc. Zool. 3: 176. Type: ♀, Aru. Neotype: ♀, Makassar, Celebes, with a handwritten label "*Bracon pallifrons* Sm." (Oxford).

SIGALPHOGASTRA PATROUS (Cameron), comb. nov.
Iphiaulax patrous CAMERON (1903). J. Str. Roy. As. Soc. 39: 106. Type: ♀, Borneo (BM 383).

SIGALPHOGASTRA RUBRILINEATUS (Cameron), comb. nov.
Iphiaulax rubrilineatus CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 151. Type: ♀, Dunbrody, Cape Colony (BM 380).

SIGALPHOGASTRA RUGIFRONS (Smith), comb. nov.
Bracon rugifrons SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 125. Type: ♀, Sarawak, Borneo (Oxford).

SIGALPHOGASTRA SADYATES (Cameron), comb. nov.
Iphiaulax sadyates CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 108. Type: ♂, Santubong, Borneo (BM 372).

SIGALPHOGASTRA SHELFORDI (Cameron), comb. nov.
Iphiaulax shelfordi CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 103. Type: ♀, Kuching, Borneo (BM 384).

SIGALPHOGASTRA SORANUS (Cameron), comb. nov.
Iphiaulax soranus CAMERON (1905). J. Str. Brit. Roy. As. Soc. 42: 26. Type: ♀, Matang, Borneo (BM 371).

SIGALPHOGASTRA SYLEUS (Cameron), comb. nov.
Iphiaulax syleus CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 198. Type: ♀, Kuching, Borneo (BM 386).

SIGALPHOGASTRA 12-FASCIATUS (Cameron), comb. nov.
Iphiaulax 12-fasciatus CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 154. Type: ♀, Dunbrody, Cape Colony (BM 381).

Genus IPHIAULAX Foerster

Iphiaulax FOERSTER (1862). Naturh. Ver. Rheinlande Verh. 19: 234.
Type: *Bracon impostor* Scopoli. By monotypy and original designation.
Synonyms: *Ipobracon* Dalla Torre, *Digonogaster* Viereck, *Monogonogastra* Viereck, *Iphiaulax* Fahringer.
Distribution: Worldwide.

The species included in this genus have a midbasal triangular area on the second tergite, but no carinæ that converge apically. The scape is short, about 1.0 to 1.5 times as long as its diameter. The following species originally described in *Bracon* are now transferred to *Iphiaulax*.

IPHIAULAX BELLICOSUS (Smith).
Bracon bellicosus SMITH (1860). J. Proc. Linn. Soc. Zool. 4: 65. Type: ♀, Makassar, Celebes (Oxford).—SZEPLIGETI (1901). Termes. Fuzetek. 24: 367. (comb. nov.) *Iphiaulax*.—SZEPLIGETI (1906). Ann. Mus. Nat. Hung. 4: 564. (comb. nov.) *Ipobracon*.

IPHIAULAX DECEPTOR (Smith), comb. nov.
Bracon deceptor SMITH (1860). J. Proc. Linn. Soc. Zool. 4: 65. Type: "♀" = ♂, Makassar, Celebes (Oxford).

IPHIAULAX DEESAE (Smith), comb. nov.
Bracon Deesae CAMERON (1902). J. Bombay Nat. Hist. Soc. 14: 433. Types: ♂, ♀ Deesa, India (London); Lectotype: ♀, Deesa, India, bearing a handwritten label "*Bracon deesaensis* Cam." (BM 434).—DOVER (1925). Ent. Mitt. 14: 39. (comb. nov.) *Glyptomorpha*.—AYYAR (1928). Mem. Dept. Agri. India Ent. Ser. 10 (3): 35. (comb. nov.) *Stenobracon*.

IPHIAULAX DODONAEUS (Cameron), comb. nov.

Bracon dodonaeus CAMERON (1899). Mem. Proc. Manch. Lit. Philos Soc. 43: 75. Type: ♀, Khasia Hills, India (Oxford).

IPHIAULAX FLORALIS (Smith), comb. nov.

Bracon floralis SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 12. Type: ♀, Sarawak, Borneo (Oxford).

IPHIAULAX INSINUATOR (Smith), comb. nov.

Bracon insinuator SMITH (1858). J. Proc. Linn. Soc. Zool. 3: 24. Type: ♀, Makassar, Celebes (Oxford).

IPHIAULAX KHASIANUS (Cameron), comb. nov.

Bracon khasianus CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 72. Type: ♀, Khasia Hills, India (Oxford).

IPHIAULAX LEPCHA (Cameron)

Bracon lepcha CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 66. Type: ♀, Khasia Hills, India (Oxford).—DOVER (1925). Ent. Mitt 14: 40. (comb. nov., syn.) *Iphiaulax.*

Iphiaulax bhotanensis CAMERON (1907). Entomologist 40: 4. Type: ♀, Buxa, Bhotan (BM 412).

Iphiaulax lineaticarinatus CAMERON (1907). Ann. Mag. Nat. Hist. (7) 19: 173. Type: "♂" = ♀, Sikkim, India (BM 409).

IPHIAULAX OBSCURILINEATUS (Cameron), comb. nov.

Bracon obscurilineatus CAMERON (1911). J. Roy. Agri. Com. Soc. Brit. Guiana 1: 308. Type ♂, British Guiana (Br. Guiana Mus.). A ♂ labelled this species bearing a locality label "British Guyanan" and tagged as BM Type No. 429 in London is not the type.

IPHIAULAX OCCULTATOR (Smith), comb. nov.

Bracon occultator SMITH (1863). J. Proc. Linn. Soc. Zool. 7: 11. Type: ♀, Mysol. Neotype: ♀, Makassar, Celebes, with a hand-written label "*Bracon occultator* Sm." (Oxford).

IPHIAULAX PAUPERATUS (Cameron), comb. nov.

Bracon pauperatus CAMERON (1900). Mem. Proc. Manch. Lit. Philos. Soc. 44: 83. Type: ♀, Khasia Hills, India (Oxford).

IPHIAULAX PENETRATOR (Smith), comb. nov.

Bracon penetrator SMITH (1863). J. Proc. Linn. Soc. Zool. 7: 11. Types: ♂, Makassar; 2 ♀, Ceram and Mysol (Oxford); Lectotype: ♀, Ceram (Oxford), Paralectotypes: ♂, Makassar; ♀, Mysol (Oxford).

IPHIAULAX PERPLEXUS (Smith), comb. nov.

Bracon perplexus SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 121. Type: ♀, Sarawak, Borneo (Oxford).

IPHIAULAX PHAEDO (Cameron), comb. nov.

Bracon phaedo CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 68. Type: ♂, Khasia Hills, India (Oxford).

IPHIAULAX QUADRICEPS (Smith), comb. nov.

Bracon quadriceps SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 122. Type: ♀, Sarawak, Borneo (Oxford).

IPHIAULAX RUFUS (Cameron), comb. nov

Exolracon rufus CAMERON (1912). Ann. Soc. Ent. Belg. 56: 371. Type: ♀, Dima, Belgian Congo (Congo Museum). There is a ♀ labelled as this species and tagged as BM Type No. 551, but it has no type locality label.

IPHIAULAX SEDITIOSUS (Cameron), comb. nov

Bracon seditiosus CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 76. Type: ♀, Khasia Hills, India (Oxford).

IPHIAULAX SIMLAENSIS (Cameron), comb. nov.

Bracon simlaensis CAMERON (1899). Mem. Proc. Manch. Lit. Philos. Soc. 43: 65. Types: 2 ♀, Simla, India (Oxford and BM 368); Lectotype: ♀, Simla, India (Oxford); Paralectotype: ♀, Simla, India (BM 368).

IPHIAULAX SUSPICIOSUS (Cameron), comb. nov.

Bracon suspiciosus CAMERON (1887). J. Proc. Linn. Soc. Zool. 2: 123. Type: ♀, Sarawak, Borneo (Oxford).

IPHIAULAX TRISIGNATUS (Kirby)

Bracon trisignatus KIRBY (1884). Ann. Mag. Nat. Hist. (5) 13: 404. Type: ♀, Pasauanco, nr. Zamboanga, Philippines (BM 437).— BALTAZAR (1966). Pacific Ins. Monogr. 8, 39 (comb. nov.) *Iphiaulax*.

IPHIAULAX VAGATUS (Smith), comb. nov.

Bracon vagatus SMITH (1857). J. Proc. Linn. Soc. Zool. 2: 124. Type: ♀, Malacca (Oxford).

The following species originally described in *Iphiaulax* were also examined and believed to belong in *Iphiaulax*. Future studies might remove some of them to other genera especially some hard-to-place males.

Iphiaulax annulitarsis CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 114. Type: ♀, Kuching, Borneo (BM 394).

Iphiaulax astochus CAMERON (1902). J. Str. Brit. Roy. As. Soc. 37: 34. Type: ♀, Sarawak, Borneo (BM 348).

Iphiaulax basimacula CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 150. Type: ♀, Dunbrody, Cape Colony (BM 365).

According to Brues, 1924 (Ann. S. Afr. Mus. 24: 61), *basimacula* is a junior synonym of *Iphiaulax nataliensis* SZEPLIGETI (1901).

Iphiaulax bucephalus BRUES (1926). Proc. Amer. Acad. Art. Sci. 61: 212. Type: ♀, Natal (BM 362).

Iphiaulax burmaensis CAMERON (1907). Ann. Mag. Nat. Hist. (7) 19: 172. Type: ♀, Shwegyin, Lower Burma (BM 337).

Iphiaulax ceressus CAMERON (1902). J. Str. Brit. Roy. As. Soc. 37: 33. Type: (sex?), Matang, Borneo (BM 347, tip of abdomen damaged).

Iphiaulax coccidomaculatus CAMERON (1906). Ann. S. Afr. Mus. 5: 46. Type: ♀, Hex River, Cape Colony (S. African Mus.). The ♀ in the British Museum from this locality and labelled as this species is not the type.

According to Turner (1917) Ann. Mag. Nat. Hist. (8) 20: 213, *coccinomaculatus* Cameron is a junior synonym of *Iphiaulax plurimacula* (Brulle), 1846.

Iphiaulax decorus CAMERON (1906). Ann. S. Afr. Mus. 5: 50. Types: ♂, ♀, Hex River, Cape Colony (S. African Mus.), Lectotype: ♀, Hex River, Cape Colony (S. African Mus.).

There is a ♀ tagged as BM type No. 342 in the British Museum from "Cape" and labelled as this species, but it is not the type.

Iphiaulax dolens CAMERON (1911). J. Roy. Agri. Comm. Soc. Brit. Guiana 1: 309. Type: ♂, British Guiana (Brit. Guiana Mus.).

There is a ♂ tagged as BM Type No. 331 in the British Museum from this type locality and labelled as this species, but it is not the type.

Iphiaulax domdamiensis CAMERON (1907). Ann. Mag. Nat. Hist. (7) 10: 170. Type: ♀, Tenasserim, India (BM 338).

Iphiaulax elizens CAMERON (1905). Entomologist 38: 107. Types: ♂♀, Deesa, India (BM 352); Lectotype: ♀, Deesa, India (BM 352).

Iphiaulax erythroura CAMERON (1905). Spolia Zeyl. 3: 85. Types 2 ♀, Kandy, Ceylon; Lectotype: ♀, Kandy, Ceylon (BM 389).

Iphiaulax fletcheri CAMERON (1908). Trans. Linn. Soc. London 12: 81. Type: ♀, Red Sea (BM 367). The identification label on the specimen is *"Iphiaulax gardeneri* Cam."

Iphiaulax haluesus CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 112. Type: ♀, Kuching, Borneo (BM 360).

Iphiaulax hookeri CAMERON (1907). Ann. Mag. Nat. Hist. (7) 19: 175. Type: ♀, Sikkim, India (BM 407).—DOVER (1925). Ent. Mitt. 14: 39. (comb. nov.) *Atanycolus.*

Iphiaulax immsi CAMERON (1913). Indian For. Records (1912) 4: 107. Type: ♂, Kaluwala nr. Dehra Dun (BM 351).

Iphiaulax laertius CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 116. Type: ♀, Kuching, Borneo (BM 411).

Iphiaulax leptopterus CAMERON (1905). J. Str. Brit. Roy. As. Soc. 42: 24. Types: ♂, ♀, Borneo; Lectotype: ♀, Borneo (BM 410).

Iphiaulax levissimus CAMERON (1905). Ann. S. Afr. Mus. 5: 44. Types: ♀ ♀, Hex River, Cape Colony (S. African Mus. & Cameron Coll.); Lectotype: ♀, Hex River, Cape Colony (S. African Mus.); Paralectotype: ♀, Hex River, Cape Colony (BM 334).

According to Roman, 1912 (Zool. Bidrag, Uppsala 1: 277), *levissimus* Cameron is a junior synonym of *Ipobracon rubiginator* (Thunberg).

Iphiaulax marcotis CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 107. Type: ♂, Lingga, Borneo (BM 357, abdomen missing).

Iphiaulax matangensis CAMERON (1903). J. Str. Brit. Roy. As. Soc 39: 113. Type: ♀, Matang, Borneo (BM 393).

Iphiaulax microphthalmus BRUES (1926). Proc. Amer. Acad. Arts Sci. 61: 227. Type ♀, Butembe, Uganda (BM 364).

Iphiaulax odontoscapus CAMERON (1905). Rec. Albany Mus., Grahamstown 1: 154. Type: ♀, Dunbrody, Cape Colony (BM 356).

Iphiaulax ornaticollis CAMERON (1905). Trans. S. A. Phil. 15, pt. 4: 205. Type: ♀, Cape Colony, Dunbrody (BM 335).

Iphiaulax permutans TURNER (1917). Ann. Mag. Nat. Hist. (8) 20: 243. Type: ♀, Mylanje, Nyasaland (BM 365).

Iphiaulax portius CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 11. Type: ♀, Kuching, Borneo (BM 359).

Iphiaulax robustus CAMERON (1905). Ann. S. Afr. Mus. 5: 57. Type: ♀, Durban, Natal, Africa (S. African Mus.). There is a ♀ tagged as BM Type No. 333 in the British Museum from this type locality and labelled as this species, but it is not the type.

SZEPLIGETI (1906). Ann. Mus. Nat. Hung., p. 582. (comb. nov.) *Goniobracon.*

According to Schulz, 1911 (Zool. Annal. 4: 71) and Brues, 1924 (Ann. S. Afr. Mus. 19: 61), *robustus* Cameron is a junior synonym of *Iphiaulax martini* (Gribodo).

Iphiaulax rotundinervis CAMERON (1911). J. Roy. Agri. Comm. Soc. B. G. 1: 311. Type: ♂, British Guiana (Br. Guiana Mus.). A specimen in the British Museum tagged as Type No. 332 and bearing the locality label of "Br. Guyana" is not the type.

Iphiaulax rubrinervis CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 152. Type: "♀" = ♂, Dunbrody, Cape Colony (BM 345).

Iphiaulax rufithorax BINGHAM (1909). Tr. Zool. Soc. London 19: 179. Type: "♂" — ♀, Ruwenzori (BM 391).

According to Roman, 1910 (Ent. Tijds., p. 114), *rufithorax* Bingham is a junior synonym of *Bathyaulax cyanogaster* Szepligeti, 1901.

Iphiaulax sadongensis CAMERON (1906). J. Str. Roy. As. Soc. Sing. 46: 105. Type: ♀, Borneo. Neotype: ♀, Sumatra (BM 369).

Iphiaulax spilonotus CAMERON (1904) 1905. Rec. Albany Mus. Grahamstown S. Afr. 1: 165. Type: ♂, Brak Kloof, S. Africa (BM 344).

Iphiaulax stramineus CAMERON (1907). Ann. Mag. Nat. Hist. (7) 19: 172. Type: ♀, Haundraw Valley, Tenasserim, India (BM 340). —DOVER (1925). Ent. Mitt. 14: 40. (syn.) — *Campyloneurus trichionotus* Cameron.

Iphiaulax tenasserimiensis CAMERON (1907). Ann. Mag. Nat. Hist. (7) 19: 176. Type: ♀, Tenasserim (BM 370). The identification label on the specimen is "*Bracon tenasserimensis* Cam."

IPHIAULAX TURNERI Baltazar, nom. nov.

Iphiaulax transiens TURNER (1918). Trans. Ent. Soc. London, p. 95. ♂, ♀. Type: ♀, Queensland, Australia (BM 366). Name preoccupied by Szepligeti (1904). Ann. Mus. Nat. Hung., p. 173.

Iphiaulax trichiosoma CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 118. Type: "♀" = ♂, Kuching, Borneo (BM 398).

Iphiaulax varicoll s CAMERON (1909). Arch. Mat. Naturv. 33 (10): 6, 7. Types: ♂, Cape Colony (Berlin Mus.); ♀, Kapland (BM 354). Lectotype: ♀, Kapland (BM 354).
Iphiaulax wallacei CAMERON (1903). J. Str. Brit. Roy. As. Soc. 39: 108. Type: ♀, Kuching, Borneo (BM 358).
Iphiaulax whitei CAMERON (1904). Rec. Albany Mus. Grahamstown S. Afr. 1: 165. Types: ♂, ♀, Brak Kloof Farm, S. Africa; Lectotype: ♂, Cape Colony (BM 343).

There are five specimens in the British Museum that bear type labels but there is no proof that the handwritten names on them have ever been published:

Barthasis ruficeps Cameron. This is a *Sigalphogastra*.
Specimen: ♀, Sarawak, Borneo, tagged as BM 509.
Bracon tricolor Smith. This is an *Iphiaulax*.
Specimen: ♀, Sarawak (Oxford).
Eurytyrymus ruficollis Cameron. This is a *Bracon*.
Specimen: ♀, tagged as BM 214.
Iphiaulax rampalicnsis Brues. This is an *Iphiaulax*.
Specimen: ♀, Rampala, tagged as BM Type No. 363.
Lissobracon nitidus Cameron. This is a *Eunabracon*.
Specimen: ♀, Borneo, tagged as BM 552 (abdomen missing). It agrees with the color description of *Lissobracon forticornis* Cameron, the type of *Lissobracon*. The type specimen of *L. forticornis* Cameron has not been located.

Subfamily ROGADINAE

PSEUDOGYRONEURON SPILONOTUS (Cameron), comb. nov.
Troporhogas spilonotus CAMERON (1905). Spolia Zeyl. 3: 93.
Type: ♀, Peradeniya, Ceylon (BM 222).

MEGARHOGAS MACULIPENNIS (Cameron), comb. nov.
Troporhogas maculipennis CAMERON (1905). Spolia Zeyl. 3: 94.
Type: ♀, Kandy, Ceylon (BM 224).

ROGAS LATERALIS (Cameron), comb. nov.
Troporhogas lateralis CAMERON (1905). Spolia Zeyl. 3: 95.
Type: ♀, Peradeniya, Ceylon (BM 227).

There are two specimens that are considered as rogadines and are tagged with British Museum Type Numbers; the manuscript names on them have never been validated by Cameron.

Eurytyrymus marginicollis Cameron. This is a *Rhaconotus*.
Specimens: ♂, ♀, Borneo, tagged as BM Type No. 215.
Onocophanes ruficaudis Cameron. This is a *Rhaconotus*.
Specimen: ♀, Borneo, tagged as BM Type No. 212.

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EFFECTS OF GAMMA RADIATION ON PEANUTS, ONIONS, AND GINGER

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ONE PLATE

Food irradiation has reached a stage wherein the potential application of ionizing energy to preserve food is presently possible. This method has shown great promise in the preservation of perishable foods such as fresh and dried foods through the destruction of microorganisms, sprout inhibition, delay of ripening, and killing or sterilization of insects that generally infest dried foods including cereal grains.

In the Philippines, the climatic conditions, food handling and storage practices are such that most foodstuffs like tubers and root bulbs develop sprouts and/or allow mold growth, while dried food products such as grains, oilseeds, beans and dried fruits are attacked by insects, mites, fungi and other spoilage agents. This situation has created heavy losses in our local food supply. Food irradiation presents possibilities of minimizing such losses.

Among the important root crops produced locally are onions, ginger, and peanuts. These crops particularly onions are seasonal. Farmers have to dispose them off at very low cost during glut seasons since they easily undergo spoliage. Serious losses occur from sprouting and rotting which generally set in within a month at ambient temperature storage.

Prestorage irradiation of onions has been demonstrated to be an efficient means for controlling sprouting over a period of months [Brownell *et al* (1954), Dallyn and Sawyer (1955 and 1957), Hori *et al* (1964), Kahan and Temken (1968), and Sawyer and Dallyn (1965)]. Gamma irradiation could be a useful means of inhibiting sprouting and/or spoilage in the local varieties of onions. A review of available literature showed no reports on postirradiation studies made on local ginger and onions.

The potential use of irradiation to solve sprouting and rotting problems in potatoes was recognized several years ago. Early

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researchers along this field were reported in Canada, the United States, and U.S.S.R., and lately in Norway, France, Poland, Japan, and Israel [Errington and MacQueen (1961), Gardner and MacQueen (1965)]. To date, the United States, Canada, and Israel have cleared irradiated potatoes for human consumption.

This study aims to determine the effects of gamma radiation on local varieties of onions, ginger, and peanuts and to develop methods for extending the storage life of these food crops. Such methods could be the basis for pilot plant work and eventually for commercial adoption.

MATERIALS AND METHODS

Peanuts.—A preliminary study on the effect of gamma radiation on shelled peanuts was conducted. Results of this preliminary investigation established the irradiation doses that were utilized in the subsequent trials that involved the effect of ambient temperature storage on the quality of the irradiated samples.

For the preliminary trials, shelled peanuts free from unsound kernels were packed in multiwall paper bags and representative batches were gamma-irradiated with the Cobalt 60 facility of the Philippine Atomic Research Center using the following doses of 0, 50, 75, 100, 125, 150, 175, and 200 Kr. The samples were analyzed for moisture, free fatty acid, thiobarbituric acid values (test for rancidity), total plate count (TPC), and mold and yeast counts. Toasted samples were evaluated for organoleptic properties using the Hedonic Rating Scale [Pilgrim and Peryam (1958)].

Based on the results obtained from the preliminary trials, the doses utilized for storage studies of the shelled peanuts packed in multiwall paper bags were 0, 50, 100, and 150 Kr. The Cobalt 60 facility of the Atoms in Action Exhibits sponsored by the U.S. Atomic Energy Commission (AEC) in cooperation with the National Science Development Board was used in the irradiation of the samples in February 1969. The experimental products were stored at room temperature (27–30°C) for a period of 10 months.

Composite samples were tested initially for organoleptic properties and analyzed for moisture, free fatty acid, aflatoxin, TPC and mold and yeast counts. The stored samples were

tested for these same properties monthly for the first 8 months of storage and on the 10th month of storage.

The following methods were used in the analyses of the experimental samples; moisture and free fatty acid (expressed as oleic acid)—The Association of Official Agricultural Chemist (1965); rancidity—thiobarbituric acid (TBA) method of Tappel *et al* (1957); Aflatoxin—method of Pons Jr. *et al* (1965); and total plate count (TPC) and mold and yeast counts—APHA method.[1]

Sensory evaluation made during storage of the irradiated and unirradiated samples were carried out by a panel of six selected members. A score sheet on preference tests of food samples prepared by the Technical Committee of the Food and Nutrition Research Center (FNRC) and approved by the Office of the Statistical Coordination and Standards, National Economic Council was used. It consists of descriptive terms with corresponding numerical scores; desirable—10, 9; acceptable—8, 7; neutral (neither like nor dislike)—6, 5; objectionable—4, 3; and unacceptable—2, 1. Analysis of variance was used in the statistical evaluation of the results of the organoleptic tests to determine the effect of storage period on the quality of the product.

Onions.—Selected mature bulbs of two onion varieties (red creole and white variety) were divided into lots of about 2 kg. each, packed in coarse-woven *sinamay*[2] bags and exposed to the gamma irradiation facility of the U.S.A.E.C. Atoms in Action Exhibit in February 1969. The following radiation doses were used: 0, 5, 10, and 15 Kr. The samples were placed in uncovered carton boxes and stored in an improvised shed to simulate storage practices in the rural areas. Composite samples were evaluated initially as is for organoleptic properties using the FNRC preference score sheet. Sensory evaluation of the samples was done monthly up to the time when almost all of the samples appeared rotten and/or sprouted.

Ginger.—Mature rhizomes of ginger (Hawaiian variety) were divided into lots of 2 kg each packed in the same way as onions and exposed to the gamma irradiation facility of the U.S.A.E.C. Atoms in Action Exhibit. Radiation doses

[1] Recommended methods for the microbiological examination of foods. Publication Office of APHA, Inc., 1790, Broadway, New York.

[2] Coarsely woven cloth material from abaca fibers.

received by representative lots were 0, 4, 8, and 12 Kr. Storage conditions and examinations made on the experimental products were the same as those described for onions.

RESULTS AND DISCUSSION

Results of analysis made on the preliminary trials with peanuts are given in Table 1. The moisture values of the experimental samples did not differ much from one another. Negative results were obtained for TBA test indicating that irradiation at the dose levels used did not cause immediate auto-oxidation of the fat content of the sample. Fat oxidation due to irradiation has been reported to proceed generally via a free radical mechanism with removal of a hydrogen from a methylene group to a double bond of the fatty acid chain [Ingold (1962)]. The direct formation of free radicals in the alkyl chain of the fatty acid plays a great part in the formation of volatile compounds that can cause characteristic off-flavors in irradiated foods rich in fats and proteins [Forss *et al* (1966)]. At the dose levels used no off-flavors were observed in the irradiated peanuts. Free fatty acid values showed very slight changes up to 150 Kr. However, with higher doses of 175 and 200 Kr, free fatty acid content increased to about 5 to 6 times that obtained for the irradiated samples.

The mean acceptability scores for texture, flavor and appearance received by the experimental samples are also given in Table 1. Values indicate that all samples were within the range of acceptable products although they could not be classified as highly acceptable. Higher mean scores for all the qualities tested were, however, obtained for peanut samples irradiated at 50, 75, and 150 Kr. Irradiation at the dose levels used did not affect markedly the organoleptic properties of the peanuts.

Results of microbiological tests showed decrease in TPC and mold and yeasts counts as irradiation dose was increased. At 75 Kr and above, mold and yeasts counts were negative. TPC was 50 colonies per gram for samples irradiated at 150 and 200 Kr.

Based on the results obtained in the preliminary tests, irradiation doses used in the subsequent storage trials were 0, 50, 100, and 150 Kr. Moisture and free fatty acid values increased

TABLE 1.—*Effect of gamma-irradiation on some of the physical, chemical and microbiological properties of irradiated peanuts.*

Radiation dose Kr	Moisture	TBA O D	Free fatty acid	T P C col gm	Yeasts and mold counts co gm	Acceptability scores (mean)		
						Eye appeal	Palatability	Texture
	Per cent		*Per cent*					
0	4 58	0	0 53	550	600	5 83	6 17	6 50
25	4 28	0	0 20	550	200	5 67	5 83	6 17
50	4 52	0	0 65	570	0	7.00	7 00	7 33
75	4 02	0	0 99	100	0	7.00	7 17	6 50
100	4 02	0	0.77	100	0	5 83	5 67	5 83
125	4 14	0	0 70	—	—	6 17	6 17	6 17
150	4 13	0	0.81	50	0	6 83	7 33	7.17
175	4 19	0	3 31	—	—	6 60	6.33	6 50
200	4 03	0	2 36	50	0	5 83	5 83	6.00

(—) not determined due to unavoidable circumstances
Hedonic rating scale 9, like extremely, 8, like very much, 7, like moderately, 6, Like slightly; 5, neither like nor dislike, 4, dislike, slightly, 3, dislike moderately; 2, dislike very much, 1, dislike extremely.

slightly during storage (Table 2). A decrease in TPC and mold and yeast counts was observed as the irradiation dose increased (Table 3). For the 1st and 2nd months of storage, TPC and mold and yeasts counts for most samples were comparatively lower than their initial counts. However, on the 3rd month up to the 10th months an increase in count was observed for samples with positive initial counts. Irradiation at 100 and 150 Kr prevented mold and yeast growth. Mold and yeast counts were negative for samples irradiated at dose levels even up to the end of the experimental period.

TABLE 2.—*Moisture and free fatty acid contents of experimental peanut samples during storage at room temperature for a period of 10 months.*

Test and irradiation dose	Storage period (month)									
	0	1	2	3	4	5	6	7	8	10
Moisture (per cent)										
0 Kr	4 43	4 68	4 41	5 02	5 3	6 35	6.10	6 47	5 74	6 00
50 Kr	4 54	4 67	4 47	4 95	5 82	6.95	6 07	6 30	5 55	6 02
100 Kr	4 45	4 36	4 20	4.88	5 70	7.04	5 50	6 49	5 54	5 69
150 Kr	4 65	4 69	4 35	4 76	4.76	7 34	5 84	6 53	5 45	5 79
Free fatty acid (per cent										
0 Kr	0 12	0 23	0 23	0 32	0 35	0 37	0 30	0 36	0 30	0 36
50 Kr	1 15	0 24	0 36	0 46	0 30	0 38	0 38	0 33	0 33	0 51
100 Kr	0 15	0 38	0 46	0 41	0 31	0 31	0 46	0 36	0 33	0 47
150 Kr	0 18	0 22	0 33	0.33	0 33	0 32	0 38	0 41	0 46	0 56

TABLE 3.—*Results of aflatoxin and microbiological tests made on the experimental peanut samples during storage at room temperature for a period of 10 months.*

Microbiological tests and dose treatments	Storage period (months)									
	0	1	2	3	4	5	6	7	8	10
Total plate count (Colonies/gm)										
0 Kr	240	10[illegible]	90	200	250	200	100	400	150	300
50 Kr	160	40	40	200	200	150	100	240	250	[illegible]
100 Kr	70	10	20	200	200	200	100	150	200	150
150 Kr	50	10	10	100	100	200	100	150	200	100
Mold and yeast count (Colonies/gm)										
0 Kr	50	20	30	150	100	100	100	100	100	100
50 Kr	10	0	10	10	10	10	10	10	10	10
100 Kr	0	0	0	0	0	0	0	0	0	0
150 Kr	0	0	0	0	0	0	0	0	0	0
Aflatoxin (p.b.p.)										
0 Kr	(—)	(—)	(—)	(—)	9.9	6.5	traces	4.7	8.7	7
50 Kr	(—)	(—)	(—)	(—)	7.7	4	1	(—)	6.5	(—)
100 Kr	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
150 Kr	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)

(—) Negative, p.p.b., parts per billion.

Aflatoxin tests for all samples gave negative results up to the 3rd month (Table 4). However, on the 4th month and up to the end of the experimental period, unirradiated samples gave positive results. The 50 Kr samples were positive for aflatoxin on their 4th, 5th, 6th, and 8th months. It is apparent that aflatoxin-producing organisms in the unirradiated sample may have survived the 50 Kr dose irradiation treatment and became active on the 4th month with the subsequent production of small amounts of the toxin. These samples can, however, be considered only slightly contaminated. Their aflatoxin content is still within the limits allowable in food products which was designated by the American and Canadian Food and Drug Administration as 30 ppb [Campbell ((1967)]. In a way, irradiation at dose levels of 100 and 150 Kr controlled aflatoxin contamination during the 10 months experimental period. It should be noted that the peanuts used in this study were of the best quality, freshly harvested and dried to a moisture content of about 4.5 per cent before irradiation. These conditions plus proper packaging to eliminate as much as possible exposure to contaminants contributed to the good keeping quality of the experimental products.

TABLE 4.—*Mean acceptability scores of experimental peanut samples stored at room temperature for 10 months.*

Qualities rested and dose treatments	Storage period (months)									
	0	1	2	3	4	5	6	7	8	10
Eye-appeal										
0 kr	8.67	7.50	7.33	7.17	7.00	7.33	5.83	7.17	6 00	6 83
50 kr	8 83	7 33	7.16	7.17	6.33	6.67	6.50	6.33	6 33	5 67
100 kr	8 83	7.33	7.00	6 50	6.17	7.00	7.00	6.17	6 33	6 0
150 kr	8.67	7.33	7.33	6 17	6 83	7 17	6.83	5.67	6 00	7 00
Palatability										
0 Kr	8.67	7.00	7 33	7.00	5 83	7.17	5.83	6.33	6.50	5.83
50 kr	8 33	7.00	7 00	7.17	6.67	6.67	6.00	6 33	6.50	5 83
100 kr	8 33	7.33	7.16	7.00	5.33	6.33	6.50	6.33	6.50	6 67
150 kr	8 17	7.00	7 00	6.17	6.17	6.83	5 67	5 50	5.83	6 50
Texture										
0 kr	8.67	5.30	7.16	6.83	6.00	6.33	5 17	6.67	6.17	6 17
50 kr	8.33	5.50	6.83	7.33	6.67	5 33	6.33	6 17	6 67	6 0
100 kr	7.83	6.67	7.16	7 17	5.67	5.00	6 50	6.17	6.33	6 33
150 Kr	7.67	6.50	6.83	6.33	6.83	6.09	6.00	6.00	6.17	6 33

The mean acceptability scores received by the various samples during the 10 months storage period are given in Table 5. Initial evaluation of the toasted peanuts, showed that irradiation at the levels used did not affect significantly the texture, palatability and eye-appeal of the peanut. A decrease in acceptability scores was, however, noted on all samples during storage. Analysis of variance of the acceptability scores showed that significant differences between storage periods occurred within samples.

Results on postirradiation studies made on ginger and onions showed that irradiation markedly improved the keeping quality of these crops in terms of sprout inhibition and control of rot decay. The gamma rays from the Co 60 facility, which are similar in nature to x-rays, easily penetrate the root bulbs, and the instantaneous interaction of the gamma rays with the very sensitive germination cells comprises the antisprouting action, i.e. the germination cells are unable to divide following exposure to suitable doses of radiation [Errington and MacQueen (1961)].

Sprouting was already evident in some of the unirradiated samples after one month storage and the number of sprouted onion bulbs and ginger roots increased during storage. Figures 1, 2, and 3 illustrate the inhibiting effect of irradiation on the

sprouting of onions and ginger. The pictures were taken on samples stored for 4 months. Irradiated samples did not show incidence of sprouting even up to the 6th month of storage.

Rotting was observed on both irradiated and unirradiated onion samples on the 3rd month but was considerably more in the unirradiated controls. Rot decay has always been associated with microbiological attack. Radiation through its fungicidal properties has been demonstrated to enhance storage life of some perishable food [Clarke (1968)]. The greatest potential advantage of gamma radiation as a fungicidal treatment is penetration of tissues, making a therapeutic treatment of the infected host possible [Sommer and Maxie (1966)]. The pathogen growing within the host tissue is inactivated or its growth delayed sufficiently to permit increased time for marketing or reduce losses during marketing periods. Both pathogen and host are subjected to the damaging events associated with irradiation.

Unirradiated samples of the white onion variety had to be discarded after the 3rd month as they were no longer acceptable. Sprouting and rot decay were very much evident in these samples. Rotting was characterized by the exudation of a watery odoriferous material from the neck of the onion, blackening, and softening of its internal portion. The red creole variety of onions was observed to have better keeping qualities and was more resistant to rot than the white variety.

Ginger samples, whether irradiated or unirradiated showed marked shrivelling during storage. The cut surfaces in some parts of the tubers may have contributed much to this undesirable effect brought about by the rapid evaporation of moisture in these cut areas.

Table 5 shows results of sensory tests made on onion and ginger samples before and during storage. Irradiation at the dose level used did not affect the acceptability of these crops as shown by their initial acceptability scores. No significant difference in acceptability was noted between the samples tested. However, analyses of variance on acceptability scores indicated a significant difference within samples stored at different periods. The unirradiated ginger and onions (red creole variety) stored for 5 months and ginger samples irradiated at 10 Kr and 15 Kr doses stored for 6 months had low

TABLE 5.—*Mean acceptability scores of experimental ginger and onion samples during shed storage.*

Trials	Storage period (months)						
	0	1	2	3	4	5	6
Ginger							
0 Kr	7.50	6 17	7 33	5.17	6.67	4.33	
5 Kr	6.33	6.17	5.83	7.50	7.00	6.17	7.50
10 Kr	7.82	7.50	5 33	6.17	7.33	7.50	4 00
15 Kr	6.33	6.33	4.67	7.50	5.00	6.83	4 00
Onions (*white*)							
0 Kr	6.50	8.00	7.67				
4 Kr	7.50	7.33	8.17	6.17	6.17	6.83	8 00
8 Kr	6.50	7.00	7.50	6.83	8.17	8.00	7 67
12 Kr	8.00	7.50	6.83	7.17	6.83	6.67	7 67
Onions (*red*)							
0 Kr	7.50	7.83	5.67	7.33	7.17	3.30	
4 Kr	6.33	7.83	8.00	6.17	6.50	7.67	7.50
5 Kr	6.83	7.17	5.50	6.67	7.17	6.00	7 00
12 Kr	7.50	7.50	4.33	6.67	6.83	5.20	5.50

acceptability scores. It is apparent that 4 Kr and 5 Kr irradiation dose treatment for onions and ginger, respectively, are sufficient to prevent sprouting. It is also possible that lower dose treatments may have inhibiting effect on sprouting. It is important to determine the optimum effective dose, because if the process is to be used on an industrial scale, the cost of treatment will depend much on the dose needed.

SUMMARY

Preliminary trials conducted on shelled dried peanuts showed that irradiation doses of 50 to 200 Kr did not affect the acceptability and TBA values of the peanuts. Microbial counts decreased as irradiation dose increased. Postirradiation studies indicate that aflatoxin contamination was controlled at dose levels of 100 and 150 Kr during the 10 months storage period. Acceptability scores for all samples decreased while free fatty acid and moisture values increased slightly during storage for 10 months at room temperature.

Postirradiation studies made on onions (red creole and white variety) and ginger (Hawaiian variety) showed that irradiation doses of 4 to 15 Kr inhibit sprouting in these crops. However, rot decay was not fully controlled although incidence was less for the irradiated samples. It is indicated that irradiation dose treatment of 4 Kr and 5 Kr for local varieties

of onions and ginger respectively, is sufficient to prevent sprouting. However, the effects of lower dose treatment of sprout inhibition needs further investigation. It is important to determine the optimum effective dose, since the economics of the process depends very much on the dose treatment.

ACKNOWLEDGMENT

The authors are grateful to the International Atomic Energy Agency for financial assistance; to Miss Lourdes A. Salamat, Miss Rosario H. Tanchuco, and Mrs. Milagros Gopez for their technical assistance in the analysis of the experimental samples; to Mr. Servando Garma for the statistical evaluation of data; and to the senior members of the Food Research Laboratory and the Technical Committee of the FNRC for their valuable comments and suggestions in the preparation of the manuscript.

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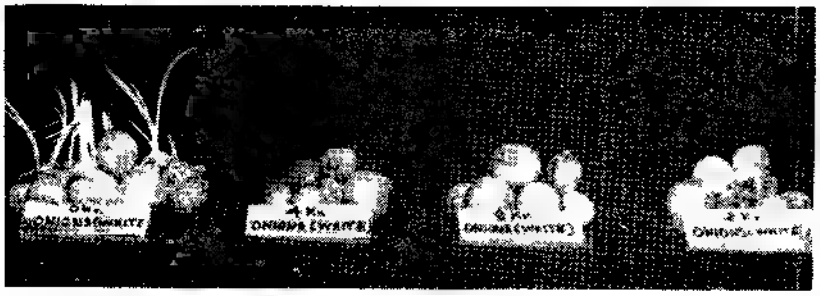
ILLUSTRATIONS

PLATE 1

FIG. 1. Effect of gamma-radiation on sprouting of onions (white variety) after 4 months in shed storage.

2. Effect of gamma-radiation on sprouting of onions (red variety) after 4 months in shed storage.

3. Effect on gamma-radiation on sprouting of ginger after 4 months in shed storage.



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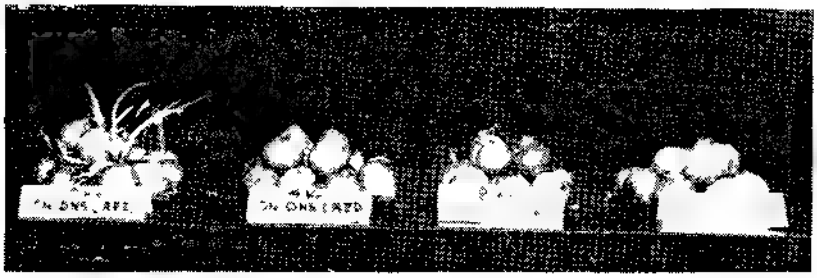

2

3

PLATE 1

ON THE TAXONOMY OF RANDIA LONGIFLORA SENSU HOOK. F. (NON LAMK.) (RUBIACEÆ)

By C. R. Babu and B. Pramanik
Central National Herbarium, Howrah-3.

TWO TEXT FIGURES

Hooker f. [Fl. Brit. Ind. 3 (1880) 111] reduced *Griffithia siamensis* Miq.[—*Randia siamensis* (Miq.) Craib; *Webera siamensis* (Miq.) Kurz] and *Stylocoryne bispinosa* Griff. [—*Randia bispinosa* (Griff.) Craib; *Webera bispinosa* (Griff.) Kurz] to the synonymy of *Randia longiflora* Lamk. [—*Posoqueria longiflora* Roxb.; *Webera longiflora* (Lamk.) Kurz], along with a few other synonyms, viz. *Randia scandens* (Bl.) DC.; *Griffithia curvata* Kurz; *Webera scandens* Roxb.; *Tocoyena scandens* Bl., etc. This view has been adopted by subsequent authors dealing with this group of plants [*vide*, Ridley, Fl. Malay Penin. 2 (1923) 73; Kanjilal *et al*, Fl. Ass. 3 (1939) 58; Parkinson, For. Fl. Andam. Isl. (1923) 190], until Craib (Fl. Siam. Enum. 2 (1932) 99, 103, 111) reinstated *R. siamensis* (Miq.) Craib, *R. bispinosa* (Griff.) Craib and *R. longiflora* Lamk. as distinct taxa.

A critical study of the material of *R. longiflora* Lamk. and *R. siamensis* (Miq.) Craib available at the herbarium of CAL and careful analysis of the original descriptions of *R. longiflora* Lamk., *R. bispinosa* (Griff.) Craib and *R. siamensis* (Miq.) Craib, do show constant distinguishing characters which justify in maintaining them as distinct taxa. These three species, no doubt, are closely related and indeed confused in the herbaria, but can be distinguished in the following way:

1. Corolla-tube 1.5 to 4 cm long; calyx-limb 0.5 to 0.6 cm long; style 1.5 to 4 cm long (incl. stigma) . . . . . 1. *R. longiflora*
   Corolla-tube 0.5 to 0.6 cm long; calyx-limb 0.3 to 0.35 cm long; style ± 1 cm long (incl. stigma) 2
2. Inflorescences and calyx glabrous or thinly ferruginous appressed-hairy on the outer calyx-lobes only; corolla-lobes oblong, longer than the tube . . . . . 2. *R. siamensis*
   Inflorescences and calyx ferrugineous, appressed-hairy; corolla-lobes obovate-oblong, as long as the tube . . . . . 3. *R. bispinosa*

1. **RANDIA LONGIFLORA** Lamk. Encycl. 3 (1789) 26 et Tab. Encycl. Meth. 1 (1792) t. 156. f. 3; DC. Prodr. 4 (1830) 386; Hook. f. in Fl. Brit. Ind. 3 (1880) 111, *pro parte* (excl. syn. *Griffithia siamensis* Miq.), *Randia siamensis* (Miq.) Craib, *Webera siamensis* (Miq.) Kurz,

Stylocoryne bispinosa Griff., *Randia bispinosa* (Griff.) Craib et *Webera bispinosa* (Griff.) Kurz; Ridley, Fl. Malay. Penin. 2 (1923) 73; Parkinson, For. Fl. Andam. Isl. (1923) 190; Craib, Fl. Siam. Enum. 2 (1932) 103.–*Tocoyena scandens* Bl. Bidjr. (1827) 980. –*Randia scandens* (Bl.) DC. Prodr. 4 (1830) 387.–*Webera scandens* Roxb. Fl. Ind. ed. carey 1 (1832) 698.–*Posoqueria longiflora* Roxb. Fl. Ind. 1 (1832) 718.–*Griffithia curvata* Kurz in Trim. Journ. Bot. (1875) 326.–*Webera longiflora* (Lamk.) Kurz, For. Fl. Brit. Burm. 2 (1877) 48.

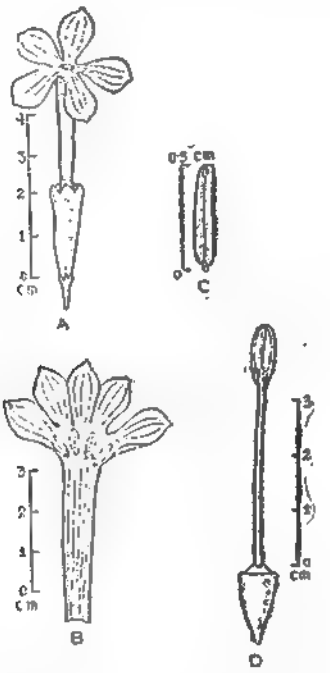

FIG. 1. *Randia longiflora* Lamk.: *A*, flower; *B*, corolla opened out; *C*, anther; *D*, gynoecium.

Distribution: India, East Pakistan, Burma, Malaysia and Siam; in India: ascending up to 660 m altitude in eastern Himalayas and Andaman and Nicobar Islands.

2. **RANDIA SIAMENSIS** (Miq.) Craib in Kew Bull. (1911) 390 et Fl. Siam. Enum. 2 (1932) 111.–*Griffithia siamensis* Miq. Fl. Ind. Bat. 2 (1856) 158 et Ann. Mus. Bot. Lugd.-Bat. 4 (1869) 130.–*Randia longiflora* sensu Hook. f. in Fl. Brit. Ind. 3 (1880) 111, *pro parte* (quoad. ref. *Griffithia siamensis* Miq. et *Webera siamensis* (Miq.) Kurz).–*Webera siamensis* (Miq.) Kurz, For. Fl. Brit. Burm. 2 (1877) 48.

Distribution: Burma and Siam.

The shorter corolla-tube, shorter calyx-limb and shorter style readily distinguish this from *R. longiflora*. Although the length of corolla-tube, calyx-limb and style is very variable in the latter species, no intermediates could be traced out.

FIG. 2. *Randia siamensis* (Miq.) Craib: *A*, habit; *B*, flower-bud; *C*, calyx opened out; *D*, corolla opened out, *E*, gynoecium.

3. **RANDIA BISPINOSA** (Griff.) Craib, Fl. Siam. Enum. 2 (1932) 99. *Stylocoryna bispinosa* Griff. Not. 4 (1854) 260.–*Webera bispinosa* (Griff.) Kurz, For. Fl. Brit. Burm. 2 (1877) 49. *Randia longiflora* sensu Hook. f. in Fl. Brit. Ind. 3 (1880) 111, *pro parte* (quoad. ref. *Stylocoryne bispinosa* Griff. et *Webera bispinosa* (Griff.) Kurz).

Type: Burma, *Griffith* 869 (K), not seen.

Distribution. Burma and Siam.

Apparently allied to *R. siamensis* (Miq.) Craib, but is recognizable by ferrugineous appressed-hairy inflorescences and outer surface of calyx.

Grateful thanks are due to Dr. M. P. Nayar, keeper, Central National Herbarium, Howrah–3, for going through the manuscript.

STUDIES ON PHILIPPINE LICHENS, II

THIN-LAYER CHROMATOGRAPHIC STUDY OF THE CONSTITUENTS OF SOME LICHEN SPECIES

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and

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ONE PLATE

Lichens received much attention because of the antibiotic activities exhibited by some lichen thalli and their extracts. This activity is due to several constituents, particularly d-usnic acid present especially in the *Usnea, Evernia,* and *Parmelia* species [Hale (1961)]. More than 80 other compounds which belong to the depsides, depsidones, dibenzofuranes and lactone-carboxylic acids have been isolated from lichens. The qualitative and quantitative differences in the constituents of lichens may be used to characterize many of them and are sometimes of taxonomic value [Hale (1961)].

Before 1936 lichen acids were detected only by macrochemical means which generally took much time. From 1936 to 1940 Asahina published many articles on the investigation of lichens dealing with simplified microchemical crystal tests for most of the common lichen acids [Hale (1961)]. Color, crystal, and fluorescence tests were used.

For the identification of lichen acids, especially those which present difficulties with the crystal tests, the use of partition chromatography was recently introduced. In 1956, Wachmeister published the identification of lichen acids by paper chromatography. Monji (1953), Ramaut (1953), and Hess (1958) obtained good results in the microdetection and separation of lichen substances by means of paper chromatographic method.

The first to employ thin-layer chromatography for the investigation of lichen constituents were Stahl and Schorn (1961). They used silica gel G-layers which was prepared with 0.5 N

oxalic acid instead of water. More recently, Bachmann (1963) used thin-layer chromatography for the separation and identification of the lichen constituents of the B-orcinol group.

This paper is a report on a survey of the constituents of some of the lichens in the Philippines. It presents the use of thin-layer chromatography in the separation and identification of lichen constituents by comparison with authentic compounds.

EXPERIMENTAL

Apparatus and reagents.—Thin layers are prepared by spreading silica gel G slurried with water (1:2.5) on glass plates and activated for 30 minutes at 110°.

The spreader was made from solid cylindrical stainless steel, 165 mm dia. and 150 mm long. The well is 49 mm deep and has a groove that is 95 mm long and 250 microns thick. (Plate 1.)

Plates were cut from 3 mm thick glass in varying sizes: 30 × 150 mm; 35 × 150 mm; 70 × 150 mm; 30 × 135 mm; 35 × 135 mm and 70 × 135 mm and chamfered at the Optics Section, SID.[1] The aligning tray 10 × 85 cm was made from 6 mm thick glass and mounted on a wooden board held in place by screwed aluminum edging. The spreader and plate holder were fabricated at the FMS.[2]

Chromatographic jars used are glass jars with fitted cover (dia. 87 mm, ht. 140 mm). They were lined with filter paper and filled with developing solvent to a height of 1.5 cm. Suitable capillary pipettes were used for spotting.

Seven solvent systems were used for developing the chromatograms: A Benzene/chloroform, 1:1 [Stahl (1965)], B Benzene/dioxane/glacial acetic acid, 90:25:4 [Bachmann (1963)], C n-Butanol/ethanol/water, 4:1:5 [Wachtmeister (1956)], D Butanol/acetone/water, 5:1:2 [Hale (1961)], E Butanol saturated with ammonia (using organic phase [Hale (1961)] F Hexane/diethyl ether/formic acid, 5:4:1 [Culberson and Kristinsson (1970)], and G Toluene/glacial acetic acid, 85:15 [Culberson and Kristinsson (1970)].

Materials.—The following lichens were the object of this study: *Usnea elmeri* Herre, *U. flexilis* Stirt, *U. hossei* Vain, *U. intercalaris* Kremp, *U. squarrosa* Vain, *Physcia albicans*

[1] Scientific Instrumentation Division, NIST.
[2] Fine Mechanics Section, SID, NIST.

(Pers.) Thoms., *Parmelia cetrata* Ach., *P. zollingeri* (Hepp.), *Crocynia membranacea* (Dicks) Zahlbr., *Ramalina farinacea* (L.) Ach., and *Stereocaulon* sp.

Procedure.—Five grams each of the five Usneas were cut into fine pieces. They were extracted in a Soxhlet with sulfuric ether for 15 hours, and methanol for 50 hours. The Usnea extracts were concentrated to a volume of about 10 ml by distilling the solvents on a water bath. Small amounts of the other lichens were also extracted with ether and likewise concentrated to a small volume before they were applied. With the use of capillary tube they were spotted four times on silica gel G plates at a distance of 10 mm apart and 20 mm from the bottom of the glass plate. The plates were air-dried after each application and placed in the jars previously equilibrated with the developing solvents. After allowing the solvent to travel to a distance of about 120 mm. the plates were removed and air-dried. The spots were detected by placing the plates in a jar saturated with iodine vapors or by spraying with anisaldehyde in 50-ml glacial acetic acid plus 1 ml concentrated sulfuric acid [Stahl (1965)].

Authentic samples[3] of lichen acids were chromatographed on thin layers using solvent B to determine their Rf values.

The ether extracts of the lichens under study were run using the seven solvent systems mentioned as a preliminary experiment. The spots did not separate very well in Solvents A, C, D, and E but showed good separation with B, F, and G. Thus all the extracts were developed with the three latter solvent systems. Since slight alteration of conditions affect the results, the identification of the spots was made by running authentic samples alongside the extracts on the same plate.

RESULTS

Table 1 gives the Rf values of some lichen substances.

Table 2 shows the result of the TLC of the lichens. Although the authentic lichen compounds were run along-side the lichen extracts the Rf values obtained were not included in the table because the two Rf values were identical.

Discussion.—It may be noted that usnic acid and salazinic acid were found common to the five *Usnea* species. In a previous work, Santos (1965) also found these two acids in *U. montagnei.* Stictic acid was detected in *U. flexilis, U.*

[3] Kindly furnished by Dr. T. R. Seshadri and Dr. D. H. R. Barton.

TABLE 1.—*Rf values of authentic samples of some lichen constituents.*

Lichen constituents	Rf values x 100*	Color reaction	
		Iodine vapors	Anisaldehyde
Atranorin	70–79	pink	yellow orange
Barbatic acid	36–40	yellow	orange
	51–56	yellow	orange
Chloratranorin	70–79	pink	yellow orange
Lecanoric acid	31–33	purple-yellow	orange red
Salazinic acid	13–18	blue	yellow
Stictic acid	25–30	blue	orange yellow
Usnic acid	67–73	yellow	red violet
Zeorin	56–65	yellow (black dots).	violet

TABLE 2.—*Rf values for thin-layer chromatography of lichen substances.*

Extracts	Rf values x 100 in solvent system			Corresponding lichen substances
	B	F	G	
Usnea elmeri Herre	13	11	6	salazinic acid
	30	17	16	stictic acid
	67	62	57	usnic acid
	36	22	26	unidentified
U. flexilis Stirt	8	5	0	protocetraric acid*
	16	11	6	salazinic acid
	25	ns	11	stictic acid
	71	62	57	usnic acid
U. hossei Vain	11	11	6	salazinic acid
	40,51	48,68	29,50	barbatic acid
	67	62	57	usnic acid
U. intercalaris Kremp.	6	5	0	protocetraric acid*
	14	12	6	salazinic acid
	70	69	57	usnic acid
U. squarrosa Vain	7	5	0	protocetraric acid*
	15	12	8	salazinic acid
	30	ns	15	stictic acid
	36,56	48,68	26,40	barbatic acid
	72	62	57	usnic acid
	19	ns	ns	unidentified
	23	ns	ns	unidentified
	48	ns	ns	unidentified
P. sublaevigata (Fers.) Thcrs.	56	51	43	zeorin
	75	60	64	atranorin
	17	ns	ns	unidentified
Parmelia cetrata Ach.	32	48	24	lecanoric acid
	76	ns	ns	atranorin
P. zollingeri Hepp.	70	62	60	usnic acid
	75	67	65	atranorin
	7	ns	9	unidentified
	14	22	46	unidentified
Crocynia membranacea (Dicks) Zahlbr.	64	51	45	zeorin
	71	60	60	usnic acid
Ramalina farinacea (L.)	64	61	50	homosekikaic acid
	68	64	[illegible]	usnic acid
	83	[illegible]	ns	unidentified
	43	45	[illegible]	unidentified
	49	54	31	unidentified
Stereocaulon sp.	79	ns	ns	atranorin
	49	ns	52	unidentified
	63	ns	ns	unidentified

B, benzene/dioxane/glacial acetic acid, 90:25:4

F, hexane/ethyl ether/formic acid, 5:4:1

G, toluene/glacial acetic acid, 85:15

* No authentic sample.

ns, no spot

elmeri, and *U. squarrosa*. The presence of stictic acid was also reported in some Indian Usneas; namely, *U. japonica* [Seshadri and Subramanian (1949)], *U. orientalis* [Dhar (1959)] and *U. florida* [Rangaswami and Rao (1955)].

The color that the various spots developed with iodine vapors and anisaldehyde reagent are very interesting and worthy of mention. It was observed that upon exposure of the silica gel G plates to iodine vapors for a longer period, small black dots appeared on the zeorin spots. This was seen in *C. membranacea* and *P. albicans*. Lecanoric acid, identified in *P. cetrata* developed a yellow core with purple trailing giving an effect of purplish yellow.

Atranorin was detected in a number of species such as *P. albicans, P. cetrata, Stereocaulon* sp., and *P. zollingeri*. Although the range of hRf value 70–79 of atranorin is very close to that of usnic acid 67–73 in solvent B, they gave different color reaction, which easily differentiates them. Usnic acid becomes yellow on exposure to iodine vapors and atranorin turns pink while with anisaldehyde reagent the former turns red violet, while the latter yellow orange (Table 1).

The extracts of *U. squarrosa, U. flexilis, U. intercalaris*, and *R. farinacea* gave several spots in solvent B, one of which traveled very slowly and gave a characteristic bluegreen color on exposure to iodine vapors. The Rf values ranged from 0.06 to 0.08. Comparison with the data of Santesson (1965) narrowed the identity to either fumarprotocetraric acid (0.08–0.09) and protocetraric acid (0.08–0.09). However, the TLC was repeated using Solvent D and the Rf value obtained was 0.43 (Santesson 0.43–0.45). The color of the spot in iodine vapors was also bluegreen. Thus the identification of protocetraric acid was made by comparing with Santesson's data and not with an authentic sample. Stictic acid and salazinic acid turned blue on exposure to iodine vapors.

SUMMARY

A total of 11 species of lichens endemic in the Philippines was studied. The constituents of these species were compared with authentic samples by thin-layer chromatography using three solvent systems. In this way salazinic acid, stictic acid, usnic acid, barbatic acid, protocetraric acid, zeorin, atranorin, lecanorin acid and homosekikaic acid were detected in the lichens studied.

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The authors wish to express their thanks to Dr. Mason Hale and Dr. Peter James for the identification of the lichens used, to Dr. T. R. Seshadri and Dr. D. H. R. Barton for samples of lichen acids, to the National Institute of Science and Technology, Manila for the use of its facilities and to the National Research Council of the Philippines for financial assistance.

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PLATE 1. ACCESSORIES FOR THIN-LAYER CHROMATOGRAPHY: 1, PLATE; 2, SCURRY; 3, ALIGNING TRAY; 4, PLATE HOLDER; 5, CHROMATOGRAPHY JAR; 6, SPREADER.

STUDIES ON LIGNIN DECOMPOSITION BY SOME LITTER FUNGI

By S. C. Agrawal
Department of Botany, University of Saugar, Saugor (M. P.) India

ONE TEXT FIGURE

INTRODUCTION

The soil is being annually supplied with considerable quantities of lignin. Among the major constituents of the cell walls, the most resistant to biological decomposition is, undoubtedly, lignin. It is superseded in relative quantity only by cellulose and hemicelluloses. The decomposition of this substance, however, is of fundamental importance, because in forests a huge amount of lignin is continually deposited upon the soil as wood waste. Our knowledge of the organisms that attack lignin, their decomposition and the environmental variables governing its loss is still very incomplete.

Cochrane (1958) and Falck (1923–1930) were the first to point out that basidiomycetes probably play an important part in the breakdown of this substance in forest litter. At a stage when there is extensive microbial development, there is a fairly rapid loss of both cellulose and lignin. This is accompanied by considerable activity on the part of the soil fauna, which often completely destroy the mesophyll tissues of the leaves, leaving only the vascular strands, the cuticular tissue and the toughened margins of the leaves. With the intense animal activity, the amount of black faecal material increases.

Our knowledge of the lignin decomposition is derived almost entirely from a study of the decomposition of wood or sawdust and the fungal species which have been examined in detail are commonly those associated with woody substrates [Gottlieb and Pelczer (1951) and Kremers (1959)].

Here the aim was to isolate the lignicolous fungi and to determine their capacity to utilize different ligninlike substances. The gradual change which takes place during the decomposition of ligninlike substances has also been discussed.

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Since lignin is known for its inert behaviour the study of

its decomposition present a special problem [Henderson (1960)], such as (*1*) difficulties arising from the chemical complexity of the lignin molecule; (*2*) difficulties in assaying for this substance; and (*3*) the isolation of purified lignin fractions, suitable for use as a microbiological substrate. In view of these difficulties the present work was based on some substitutes of lignin such as ferulic acid, vanillin and p-hydroxybenzaldehyde, which are believed to be related structurally to the lignin molecules [Brauns (1939), Creighton *et al* (1944), Henderson (1960), and Siegel (1956)].

MATERIALS AND METHODS

Sampling of the soil-litter.—Forest soil-litter samples from the 9″ depth were collected after the rainy season. The samples were brought to the laboratory in new polythene bags and were stored in a refrigerator till the following day, when dilutions were made.

Isolation of lignin decomposing fungi.—Waksman's (1916) dilution plate technique was used in the process of isolation. The medium used for the isolation was Waksman agar with few modifications as mentioned below:

a. Replacement of peptone by $(NH_4)_2 SO_4$ to decrease the growth rate of rapidly growing fungi.
b. Addition of tannic acid at a concentration of 0.1 per cent (w/v).

The second modification was based on the work of Bavendamm (1928) and Davidson *et al* (1938). They showed that wood rotting fungi or the lignin decreasing fungi can be detected by their reaction with tannic acid which they oxidize to a brown product. Tannic acid is somewhat toxic and inhibits the growth of most of the bacteria. Different dilutions (1:100, 1:1000 and 1:10000) of the soil-litter samples were made and streaked on the plates. The streaked Petri dishes were incubated for 8 to 10 days at 28°C. The dishes were examined after 8 days.

A total of 25 species (Table 1) was isolated out of which only 16 showed the brown color reaction around the colony. Only 10 species were selected for detailed study.

These were *Rhizopus nigricans, Chaetomium globosum, Aspergillus niger, Penicillium verruculosum, Paecilomyces varioti, Memnoniella echinata, Trichoderma viride, Alternaria tenuis, Fusarium oxysporum,* and *Rhizoctonia* sp.

TABLE 1.—*List of lignin-decomposing fungi isolated from soil-litter and their ability to oxidize tannic acid.*

	Organism	Species showing brown product
1	*Rhizopus nigricans*	*
2	*Absidia bodini*	—
3.	*Chaetomium globosum*	*
4.	*Aspergillus niger*	*
5	*A. flavus*	*
6	*A. ustus*	*
7.	*A. ochraceus*	*
8	*Penicillium verruculosum*	*
9.	*P. funiculosum*	*
10	*P. notatum*	*
11.	*Paecilomyces varioti*	—
12.	*Gliocladium roseum*	*
13	*Memnoniella echinata*	*
14	*Stachybotrys atra*	—
15	*Thielavia basicola*	*
16	*Trichoderma viride*	*
17	*Curvularia lunata*	*
18	*Alternaria tenuis*	—
19	*Helminthosporium gramineum*	*
20	*Fusarium oxysporum*	*
21	*F. longipes*	*
22	*Torula thermophila*	*
23	*Epicoccum* sp.	—
24	*Mycelia sterilia*	*
25	Black sclerotial form	*

* oxidize tannic acid. —, did not oxidize tannic acid.

Utilization of ligninlike substances by 10 selected fungal species:

Method.—The medium used here was Czapek's mineral salt with some modifications. Sucrose and ferrous sulphate ($FeSO_4$, $7H_2O$) were omitted, the latter because of its reaction with the phenolic compounds to form colored products. Broth medium, to which phenolic compounds were added, was used for noting the extent of growth of various fungi. The quantity of growth was measured in terms of mycelial weight produced.

The phenolic compounds, p-hydroxybenzaldehyde and vanillin were added at the rate of 0.01 per cent (w/v) and ferulic acid at 0.005 per cent (w/v).

Fifteen ml of the broth medium was taken in 150 ml flasks and sterilized by autoclaving for 15 minutes at 15 lbs pressure. A series of control flasks was also run in which no carbon source was added. A total of 40 flasks was taken for the 10 organisms. The flasks were inoculated with 6 mm diameter inoculum disk taken from the growing margins of potato dextrose agar culture. The flasks were incubated for 21 days at 28°C.

RESULTS

After 21 days the mycelial mat of each flask was filtered through oven dry, weighed filter papers (Whatman No. 1). After washing with distilled water oven dry weight was determined. The net value of mycelia was calculated by subtracting the weight of the filter paper. Results are shown in Table 2 and Fig. 1.

TABLE 2.—*Oven dry weight of mycelium (in mg) at 28°C after 21 days incubation in different phenolic, ligninlike substances.*

Organism	p-Hydroxy benzaldehyde (0.01 per cent w/v)	Ferulic acid (0.01 per cent w/v)	Vanillin (0.01 per cent w/v)	Control
1. *Rhizopus nigricans*	55	12	50	16
2. *Chaetomium globosum*	86	95	80	11
3. *Aspergillus niger*	98	88	92	12
4. *Penicillium verruculosum*	70	85	78	12
5. *Paecilomyces variotii*	48	21	19	12
6. *Memnoniella echinata*	69	71	62	18
7. *Trichoderma viride*	93	107	88	18
8. *Alternaria tenuis*	73	58	60	26
9. *Fusarium oxysporum*	2	38	41	8
10. *Rhizoctonia* sp.	78	82	75	20

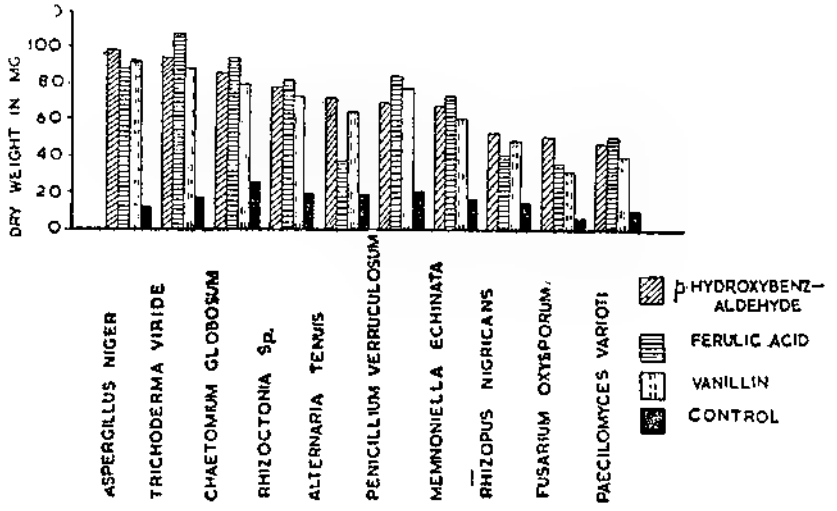

FIG. 1. Utilization of ligninlike substances in terms of mycelial growth.

In this experiment phenolic compounds constituted the sole source of carbon and were added in the medium in such low concentrations that none of the fungal species produced much

growth. From results presented in Table 2 it is clear that although the growth was very little it always exceeded that of the control flask of the same fungal species. The fungi which obtained their maximum growth in ferulic acid were *C. globosum, P. verruculosum, P. varioti, M. echinata, T. viride* and *Rhizoctonia* sp. Only four fungal species viz., *R. nigricans, A. niger, A. tenuis,* and *F. oxysporum* showed best growth on p-hydroxybenzaldehyde. None of the fungi showed their maximum growth on vanillin but most of the species utilized it almost to an equal extent. In the whole experiment *T. viride* favors its maximum growth on ferulic acid followed by *A. niger* and *C. globosum*. *P. verruculosum* showed somewhat greater growth in the control, when compared with other species. Minimum mycelial weight in the control flask was recorded for *F. oxysporum.*

The mycelial weight of the organisms in the control series was much less due to the total absence of any carbohydrate source. A glance at the table shows that the majority of fungal species utilize ferulic acid to the maximum as shown by their mycelial weights.

Evidence for utilization of ligninlike substances as a source of carbon:

In the previous experiment the mycelial development was taken as an index of utilization of the phenolic compounds by different fungi. In the present experiment the substrate utilization was measured by Chromatographic methods which revealed the total utilization of a substrate when their presence was not detected by the chromatograms run from the culture filtrates after varying intervals of time.

Method.—Twenty ml broth of the basal medium was taken in a 150 ml conical flask. Two flasks for each fungal species and for each phenolic compound were prepared. A total of 60 flasks was autoclaved at 15 lbs pressure for 15 minutes. After autoclaving, the three phenolic compounds were added in the same quantity and by the same way as mentioned in previous experiment.

Each flask was inoculated with three disks (6 mm diam) cut from the growing margin of the fungi cultured on potato dextrose agar. The flasks were incubated at 28°C for 14 and 21 days. The culture filtrate of one set of flasks was analysed after 2 weeks and the rest of the flasks after 3 weeks.

The resulting fungal growth in the flask was removed by filtration and the filtrate acidified. The culture filtrate of each organism was then extracted three times with 10 ml ether. The ether was evaporated and a few drops of absolute ethanol were added to the residue to dissolve it. Phenolic compounds were detected by ascending thin-layer chromatography. The solvent used here was of the following composition [Davidson *et al* (1938)]: Benzene + Methanol + Acetic acid (45:8:4).

When the solvent rose to a height of about 10 cm, the plates were removed from the tank and dried.

The following three spraying reagents were used to trace the presence or absence of phenolic compounds in the culture filtrate initially spotted on the plates:

1. Anisaldehyde/Sulfuric acid reagent: A mixture of 5.0 ml anisaldehyde in 50.0 ml glacial acetic acid with 1.0 ml reagent grade sulfuric acid was sprayed on to the chromatogram and heated at 100 to 110°C for 5 to 10 minutes.
2. Potassium permanganate: 0.1 N potassium permanganate in sodium carbonate solution.
3. Antimony pentachloride: Two parts by volume of antimony pentachloride were mixed with eight parts by volume of carbon tetrachloride. After spraying the plates were exposed to a temperature of 120°C for a few minutes.

Out of these, antimony pentachloride was found to be the best for developing chromatograms.

During decomposition vanillin and ferulic acid are known to be converted into vanillic acid. Spots of vanillic acid were detected on the chromatograms of the culture filtrates, where vanillin and ferulic acid decomposed into vanillic acid; but in some cases vanillin and ferulic acid appeared as such indicating no decomposition. No intermediate product of p-hydroxybenzaldehyde is known until now and it does not appear on chromatograms indicating rapid utilization.

RESULTS

Table 3 shows that some of the fungi like *A. niger*, *P. verruculosum*, *M. echinata*, and *A. tenuis* left no initially added phenolic compound and showed the spots of the metabolized product (vanillic acid). This indicates the utilization of ferulic acid and vanillin after 14 days, same was the case with p-hydroxybenzaldehyde. *F. oxysporum* did not utilize

TABLE 3 — *Analysis of culture filtrate after growth of various fungi for 14 and 21 days at 28°C on mineral salt medium + phenolic compounds.*

Organism	Phenolic compound added as carbon source									
	p Hydroxybenzaldehyde (0.01 per cent w/v)		Ferulic acid (0.005 per cent w/v)				Vanillin (0.01 per cent w/v)			
			Ferulic acid + Vanillic acid				Vanillin + vanillic acid			
	14	21	14	21	14	21	14	21	14	21
1 *Rhizopus nigricans*	+	-	+	+		-	+	+	-	-
2 *Chaetomium globosum*	+	-	-	-	+		-		+	+
3 *Aspergillus niger*	-	-	-		+	+	-	-	+	+
4 *Penicillium citrinum*			-	-	+	+	-	-	+	+
5 *Fusarium oxysporum*	-	-	-	-	+	+	+			+
6 *Myrothecium verrucaria*	-	-			+	+	-		+	+
7 *Trichoderma viride*	+	-	-	-	+	+	+	-	-	+
8 *Alternaria tenuis*					+	+	-	-	+	+
9 *Rhizoctonia* sp.	-	-	+	+	-		+		-	+
10 *Botryosphaeria* sp.	-	-	-	-	+	+	+	-	-	+

14 and 21 Days of incubation.
+, Present
-, Absent.

ferulic acid even after 21 days of incubation. *T. viride, F. oxysporum* and *Rhizoctonia* sp. did not show any change in the initially added vanillin after 14 days but after the period of 21 days the presence of vanillic acid was recorded, indicating an ability to utilize vanillin at a slow rate, same was the case with *C. globosum* and *T. viride* in p-hydroxybenzaldehyde. *R. nigricans* was exceptional. It did not metabolize ferulic acid and vanillin even after 21 days. On the whole p-hydroxybenzaldehyde was found to be the most susceptible in comparison to the rest of the two phenolic compounds and it was decomposed completely within 14 days by all the fungal species except for a few species viz., *R. nigricans, T. viride* and *C. globosum* which metabolized it completely only after 21 days of incubation.

DISCUSSION AND CONCLUSIONS

The data show that there exists a wide variety of fungi which can decompose p-hydroxybenzaldehyde, ferulic acid, and vanillin. Fungi studied here were only those which could be isolated by dilution plate technique, but large number of basidiomycetes and ascomycetes which have not been included here also play an important role in the decomposition of lignin.

Here the method of isolation of lignin fungi was based on the ability of the fungi to oxidize tannic acid [McKay (1959)] and out of a total of 25 isolates only 16 fungal species were found capable of oxidizing tannic acid. To confirm further their ability, only 10 selected species were grown on the three substitutes of lignin. The data show that most of these fungi utilize p-hydroxybenzaldehyde and ferulic acid easily (shown in terms of mycelial growth). In the entire experiment, maximum growth by *T. viride* was shown on ferulic acid followed by *A. niger* on p-hydroxybenzaldehyde and *C. globosum* again on ferulic acid.

Some gungal species like *R. nigricans, P. varioti,* and *F. oxysporum* which showed their reaction with tannic acid did not show much response in the utilization of phenolic compounds. Hence these forms cannot be regarded as lignin decomposers in this respect. On the other hand *A. niger, C. globosum, P. verruculosum, M. echinata,* and *A. tenuis* utilized all the three phenolic compounds in the experiment and this property was also confirmed by their conversion of phenolic compounds in broth medium.

As tannic acid oxidation depends on the formation of quinone [Creighton *et al* (1944), Gottlieb and Pelczer (1951)] and the breakdown of the aromatic ring, this suggests the action of some enzyme system for accomplishing the process.

Davidson *et al* (1938) also reported a number of fungi which oxidize tannic acid but were weak lignin decomposers. This type of fungi which were isolated on the basis of their oxidation of tannic acid were actually not good utilizers of the resultant components. It is quite likely that these fungi which are not able to utilize the substratum alone may be depending upon the synergistic action of other organisms for the utilization of the products in natural conditions where the microorganisms are known to live in balance with each other.

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* Not seen in original.

SHORT COMMUNICATION

MODIFIED LOFTON-MERRITT STAIN FOR DIFFERENTIATING UNBLEACHED SULFITE AND SULFATE FIBERS

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Very early in the history of fiber technology, attempts were made to secure differential staining of various types of fibers by the use of different dyes or combination of dyes. Thus far, however, the results have not been very successful.

Lofton and Merritt [Snell and Biffen (1944)] reported a method for differentiating and estimating unbleached sulfite and sulfate pulps in paper and this method is being used up to the present time. The stain used consists of one part of a 2-per cent aqueous solution of Malachite green and two parts of 1-per cent aqueous solution of basic fuchsine or magenta. The two solutions are kept separately in tightly stoppered bottles and mixed together just before use.

In using the Lofton-Merritt stain, the compound stain is added to the fibers on the slide and allowed to remain 2 minutes. At the end of this period, the excess stain is removed by means of hard filter paper. Then, 3 to 4 drops of 0.1-per cent HCl solution (1 cc conc. HCl diluted to 1 liter of water) are added and left for about 30 seconds. The acid is then removed with a blotter and a few drops of distilled water added. A cover glass is then placed on top of the fibers and the slide gently placed between two pieces of blotting paper to remove any excess water.

The Lofton-Merritt stain, even when properly made and used, sometimes does not give the desired results. In testing the stain, too intense color reaction of either one of the dyes used often confuses the differentiation. Furthermore, due to the variation in the quality of the dyes, it is possible that the proportions of the two solutions as here recommended may not give the best results; hence, it is necessary to do verification tests on authentic samples, altering the proportions of the solutions until the fibers are stained the proper color. A

thorough investigation was therefore made in the Paper Laboratory of the Tests and Standards Laboratories of the NIST, and the resulting modified Lofton-Merritt stain gave a more definite differentiation of unbleached sulfite and sulfate. Equal amounts of the dyes were used in the preparation and an organic acid was added in making up the dye solutions. Different concentrations of the acid were tried but the solutions that gave the best color contrast at the acidity given in the formulas are:

Solution A:	
Basic fuchsine	0.25 g
Acetic acid	15.00 ml
Water up to	100.00 ml
Solution B:	
Malachite green	0.25 g
Acetic acid	15.00 ml
Water up to	100.00 ml

Each solution is made separately, then mixed in equal proportion as needed.

Procedure.—Disintegrate the paper and filter off loading, etc. through a small 300-mesh filter. Press a small amount of the fibers between two fingers to expel excess water. Place the sample on a spot plate and add a few drops of the newly mixed solutions A and B to the fibers. Allow the stain to remain for 2 minutes while teasing apart the fibers. This teasing is necessary to allow the stain to act evenly on all the fibers. After 2 minutes, the fibers are washed several times with distilled water to remove excess stain. The fibers are then spread thinly on the slide, and a cover glass placed over them for examination under the microscope.

Unbleached sulfite fibers produce a reddish violet color while unbleached sulfate are blue in color.

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SHORT COMMUNICATION

STORAGE LIFE OF FREEZE-DRIED NIST* ALLERGENIC EXTRACTS

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The preparation of lyophilized or freeze-dried local allergenic extracts is one significant development in the pharmaceutical field.

Studies previously conducted by Laserna *et al* (1960), on the production of local allergenic extracts of reliable nature have emphasized the need for having these extracts freeze-dried. However, when these extracts were held in the freeze-dried state and stored for prolonged periods at refrigerated conditions, the rate at which they lost activity was quite detectable. The problem therefore, of preserving them for future use without reducing their activity has come into focus.

No detailed investigation, so far, has been done on the storage life of local freeze-dried allergenic extracts. This study, therefore, is a preliminary investigation intending to put the authors' data to use as reference for further study.

MATERIALS AND METHOD

Freeze-dried extracts used in this study were those prepared by the investigators in 1965. The prepared aqueous extract was filtered through aseptic filters and the mixture was immediately distributed into vials in 5-ml volumes, followed by freeze-drying in a Stokes' freeze-drier. The vials were sealed off *in vacuo* and the activity of the extract was investigated; labeled, then stored at a refrigerated temperature of about 35°F. These allergenic extracts were restandardized to determine their actual protein nitrogen content. The methods adopted in the present investigation are described in detail and reported in previous articles by Laserna and Manalo (1966).

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The protein was precipitated from the reconstituted extract by the methods of Cooke (1947), and the total amount of protein precipitated was estimated by analysis for nitrogen by a micro-Kjeldahl (titrimetric) method of Sobel *et al* (1944). The experimental work was done in duplicate.

Results obtained were further analyzed using the "t" test [Snedecor (1946)] at 5-per cent level of significance, to determine the extent of degradation of the freeze-dried allergenic extracts.

RESULTS AND DISCUSSION

Table 1 shows the protein nitrogen content before and after storage of freeze-dried allergenic extracts from house dust and 17 local plants, and the loss of allergenic activity after 5 years at 35°F. The values shown are the average of two trials for the method used. The freeze-dried extract samples studied represent those causing allergy.

TABLE 1.—*Loss of protein nitrogen content in the freeze-dried local allergenic extracts after 5 years storage at refrigerated temperature* (35°F).

Local allergenic extracts	Protein (mg) content of extract		
	1965 mg %	1970 mg %	Loss on storage mg %
Bermuda grass [*Cynodon dactylon* (L.) Pers.]	0.110	0.105	0.005
Carabao grass (*Paspalum* sp.)	0.096	0.076	0.020
Crab grass (*Digitaria* sp.)	0.102	0.079	0.023
Dust mixture	0.094	0.084	0.010
Foxtail millet (*Pennisetum glaucum* [*P. typhoideum*] (L.) Schultz)	0.175	0.150	0.025
Johnson grass (*Polypogon procerus* Nees ex Steud.)	0.130	0.120	0.010
Kogon (*Imperata cylindrica* var. *Major* [Nees] C. E. Hubb.)	0.170	0.155	0.015
Maize (*Zea mays* L.)	0.090	0.080	0.010
Makahiya (*Mimosa pudica* L.)	0.070	0.063	0.007
Mexican Sunflower (*Tithonia diversifolia* A. Gray)	0.141	0.130	0.011
Natal grass (*Rynchelytrum repens* [Willd.] C. E. Hubb.)	0.160	0.125	0.035
Para grass (*Brachiaria mutica* [Forssk.] Stapf)	0.168	0.142	0.026
Rice (*Oryza sativa* L.)	0.170	0.140	0.030
Sugarcane (*Saccharum officinarum* L.)	0.086	0.071	0.015
Talahib (*Saccharum spontaneum* L. ssp. *arundinaceum* Hack.)	0.160	0.125	0.035
Tridax (*Tridax procumbens* L.)	0.201	0.186	0.015
Uray (*Mariscus cyperinus* [Retz.] Vahl)	0.115	0.095	0.020
Yardgrass [*Eleusine indica* (L.) Gaertn.]	0.230	0.200	0.030

Although considerable efforts have been centered on the step by step preparation of the allergenic extracts to obtain good quality freeze-dried samples and on the use of the most suitable equipment needed for the study, nevertheless, it is to be noted that the allergens showed a definite reduction of their protein nitrogen content as the period of storage

was prolonged. The decrease in the protein nitrogen content of the refrigerated allergens was not necessarily uniform, but varied with the specific allergenic extract preparation.

As shown in Table 1, the results indicate a decrease in protein nitrogen content of from 0.050 to 0.014 mg per cc, indicating a loss of from 8 to 50 per cent. This loss may be due to various adverse changes taking place in the freeze-dried extracts, such as protein denaturation or deterioration, which as yet, could not be established definitely. However, Cooke (1947) stated that ". . . while aging did not affect the total nitrogen of an extract it did influence greatly the fractions involved, the protein nitrogen and the nonprotein nitrogen, the former being converted gradually into the latter, and that as this occurred a corresponding loss in activity was evident. . ."

Table 2 shows the "t" test values for the comparison of the protein nitrogen content of the extracts before and after storage. The value of "t" obtained was more than the "t"

TABLE 2.—*T test values for the comparison of the protein nitrogen content of the freeze dried extracts before and after storage.*

Local and scientific names	T test values*
Bermuda grass (*Cynodon dactylon* (L.) Pers.)	7.0
Carabao grass *Paspalum* sp.)	1.4**
Crab grass *Digitaria* sp.)	7.0
Dust house	5.0
Foxtail millet (*Pennisetum hotentotes polystachyon*) (L.) Schultz	5.0
Java grass *Poygonum praemorsa* Neus Hacka	***
Kogon *Imperata cyndrica* (L.) Beauv.)	3.0
Mais (*Zea mays* Linn	5.0
Makahiya *Mimosa pudica* Linn.	7.0
Mexican sunflower (*Tithonia diversifolia* A. Gray)	3.0
Natal grass (*Rhynchelytrum repens* (Willd.) C. E. Hubb.)	7.0
Para grass (*Brachiaria mutica* (Forssk. Stapf.)	5.0
Rice (*Oryza sativa* Linn.)	7.0
Sugar cane (*Saccharum officinarum* Linn.	7.0
Talahib (*Saccharum spontaneum* L. subsp. *aristatum*) Hack.)	3.0
Tridax (*Tridax procumbens* Linn.)	4.0
Ulasimang Bato (*Amaranthus spinosus* Linn.)	3.0
Yard grass (*Eleusine indica* L., Gaertn.)	

* Group comparison method.
** Not significant at 5 per cent level.
*** Infinity.

value of 2.92 at 5-per cent level of significance. Hence, it may be said that, except for carabao grass, these allergenic extracts exhibited a significant variation in their protein nitrogen content, as evident in the results shown in Table 2, thereby showing the extent of reduction in protein nitrogen of the

freeze-dried extracts stored for 5 years at constant low temperature (35°F). Considering the lapse of time of 5-year storage of the extracts in the present study, it will not be surprising to note that the values obtained are much lower as compared with those obtained from the same extracts in 1965.

Since appreciable losses in protein nitrogen content were noted after 5 years, Cooke's observation, therefore, could be taken as a contributory factor in the decrease in protein nitrogen content of the locally prepared freeze-dried allergenic extracts but not conclusive to assess that the corresponding loss in activity would render the extracts ineffective, until other conditions or factors shall have been considered and confirmed.

It should be pointed out further, that although the decrease showed marked differences in protein nitrogen content, it did not render the extracts ineffective, as it was still acceptable to the allergist [1] doing clinical studies on the restandardized extracts. From the restandardized extracts dilution could still be made for test and treatment.

However, in a field where a change in activity of an extract is of particular importance to the allergists engaged in the treatment of allergic diseases, it is suggested that further detailed investigations or studies be made with particular emphasis on the factors which affect to a large degree the activity or strength of the extracts, in order to effect stability after prolonged storage. This is deemed necessary if the clinical use of the knowledge obtained in this study is intended to have local allergenic extracts available and potentially stable for human use at the appropriate time when it is most needed.

SUMMARY

Restandardization of freeze-dried NIST allergenic extracts from house dust and 17 local plants after 5-year storage at refrigerated temperature (35°F), was undertaken. Statistical analysis of the data obtained to evaluate further the extent of reduction in the protein nitrogen content of the

[1] Eleonora P. Dacanay, clinical allergist, Allergy Research Unit, Medical Research Center, National Institute of Science and Technology, Manila.

extracts, was also made, and the results are reported. The results indicate that all the protein nitrogen present during storage show losses of from 8 to 50 per cent and the significance of this was discussed. However, results of the study also tend to show that the protein nitrogen values obtained although much lower as compared with those obtained from the same extracts in 1965, were still acceptable, inasmuch as the strength of the extracts used by the allergist in her treatment was based on the recent protein nitrogen values obtained.

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The authors are grateful to Mrs. Guillermina C. Mañalac, Asst. Director of the Industrial Research Center, National Institute of Sicence and Technology for her helpful suggestions and to the Techno-Economic Staff also, of the National Institute of Science and Technology, for their technical assistance.

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SHORT COMMUNICATION

PLANTS INJURED BY AIR POLLUTANTS

By P. S. Subido, P. A. Santamaria, E. N. Alqueza, and A. C. Pizarro

Bureau of Plant Industry, Manila

ONE PLATE

INTRODUCTION

The establishment and expansion of fertilizer and other industrial factories in this country have inevitably created specific agricultural problems including the detrimental effects on the health of the population of the affected areas. Dust from cement factories have been a constant source of complaints among farmers and residents of the area. Similarly other factories have not only inflicted serious injury to plants but also caused destruction to animal fauna. Because of the irreparable injury to plants and its possible deleterious effect to human beings, measures to remedy the abnormality have attracted the attention of the National Air and Water Pollution Commission.

The case at the Lamao Experiment Station, Lamao, Bataan where a certain fertilizer factory is located is the first of its kind reported in the country. A wide range of crops including fruit trees were defoliated. Shedding of burned leaves started on the side of the trees facing the direction of the wind. The distance of the factory from the orchard where the observations were made was from 10 meters to 4 kilometers away. Depending on the susceptibility of the trees, defoliation may be either partial or entire as shown in Plate 1, figs. 1 3. Recovery of the affected trees took a long time and most of the trees failed to produce flowers. Newly developed leaves were scorched before they could mature. Isolation and other tests for pathogenicity and insects failed to yield positive relations with the abnormalities observed.

SYMPTOMS OF INJURY TO CROP PLANTS

The typical symptoms and condition of the plants affected by corrosive sulfur dioxide and hydrogen flouride gases

exhibited shrivelled, injured and burned leaves. Branches and twigs were covered with grayish deposits of fertilizer dusts and other particulates and plants affected varied from the succulent vegetables to the hardy fruit trees. (Plate 1, fig. 2.)

Injury to the different plants ranged from mere discoloration to scorching of the leaves. The margin of santol leaves after a series of exposures to fallouts first turned pale, then colorless and finally brown. The periphery of the leaves showed the first signs of burning with a tendency to cup-up. The cupped leaves accumulated emitted particulates, dusts and other gases. The presence of little moisture in combination with the particulates and dusts hastened the bleaching and scorching of the leaves while the midvein and leaf lamina near the base remained green. The bleached portion of the leaf was observed to be very brittle. All the burned leaves fell until the tree was completely defoliated resembling dead trees as shown in Plate 1, fig. 3. Continuous exposure of the trees eventually resulted in the death of the twigs and smaller branches.

The effect on sweet potato was even more devastating. This crop was considered to be the most sensitive among the plants found in the station. The leaves turned brown and water-soaked after a day exposure followed by a total collapse. On the average, a field of sweet potato was completely destroyed in 3 days in an air-polluted environment. This plant may then be considered a good bio-indicator for the presence of atmospheric pollutants.

Affected mangoes and santol trees flowered in an unusual fashion. Instead of the flowers arising from the growing points or terminal buds they sprouted anywhere from the branches or from the trunk itself. Cashew, a hardy plant suitable to the area, failed to flower as a result of its exposure to the polluted air. On the other hand, caimito and chico and the ornamental crotons were the least affected.

Leaves of affected rice plants exhibited blotchy areas of irregular shapes and sizes. These bleached patches coalesced till the whole leaf was covered. Plants so affected became stunted in growth. Production of tillers was suppressed. Corn was slightly more sensitive than rice, with leaves getting scorched at a faster rate. In coconuts, the most obvious symptoms were burning of the leaves which usually started

from the tips and margins. These scorched areas were at first small with regular outlines, later assuming a dark discoloration. The young leaves turned dark.

Affected rice leaves showed the injury of fertilizer dust and gases like sulfur dioxide and hydrofluoric acid emitted from the factory located nearby.

The extent of spread of the injury corresponds to a well defined area following the wind direction. The magnitude of destruction was less near the source of pollution and becoming more intense further away. All the plants within the circumscribed area of a mile and a half from the factory were affected in various degrees (Table 1). This type of spread corresponds to the dispersal of pollutants as shown by Smith (1968).

From field observations and laboratory examinations of affected plants, we have come to the conclusion that atmospheric pollutants from the fertilizer factory was responsible for the injuries of the different crops reported in this paper. It is an established fact that air pollutions in the form of sulfur dioxide and hydrogen flouride are toxic to a number of plants.

The absence of chemotherapeutants to be applied to counteract the effect of these oxidants indeed pose a serious problem. Ways and means should be worked but to minimize, if not entirely eliminate, the emission of toxic gases from different sources. The elimination of atmospheric pollutants has been shown to be feasible in other countries.

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TABLE 1.—*Reactions of plants to air pollutants emitted from fertilizer factories. The basis of the degree of susceptibility on the affected plants is based on the burning of the leaves.*

Very severe	Severe	Moderate	Negligible
Sweet potato (*Ipomoea batatas* Lam.) Coconut (*Cocos nucifera* L.)	Acacia (*Samanea saman* Merr.) African oil palm (*Elaeis guineensis* Jacq.)	Banana (*Musa sapientum* Kuntz) Citrus (*Citrus* spp.)	Agoho (*Casuarina equisetifolia* L.) Black pepper (*Piper nigrum* L.)
Cashew (*Anacardium occidentale*) Mango (*Mangifera indica* L.) Pechay (*Brassica chinensis* L.) Sarita (*Scaevola koetjape* (Burm. f.) Merr.)	Avocado (*Persia americana* Mill.) Bamboo (*Bambusa spinosa* Roxb.) Camachile (*Pithecolobium dulce* Roxb.) Camias (*Averrhoa bilimbi* L.) Cassava (*Manihot esculenta* Crantz) Corn (*Zea mays* L.) Duhat (*Eugenia cumini* (L.) Druce) Grapes (*Vitis* spp.) Nangka (*Artocarpus integra* Merr.) Onion (*Allium cepa* L.) Papaya (*Carica papaya* L.) Yard long, sitao (*Vigna sesquipedalis* Fruwirth) Tamarind (*Tamarindus indica* L.)	Cypress (*Cupressus* spp.) Eggplant (*Solanum melongena* L.) Guava (*Psidium guajava* L.) Kapok (*Ceiba pentandra* (L.) Gaertn.) Okra (*Abelmoschus esculentus* Moench) Pili (*Canarium ovatum* Engl.) Rice (*Oryza sativa* L.) Tiesa (*Lucuma nervosa* A. DC.) Canistel (*Pouteria campechiana* Baehni)	Caimito (*Chrysophyllum cainito* L.) Chico (*Achras zapota* L.) Croton ((San Francisco) (*Codiaeum variegatum* Blume). Papua (*Polyscias fruticosa* Harms

ILLUSTRATIONS

PLATE 1

FIG. 1. A partially defoliated cashew plant with dried flowers and dying twigs as a result of its continuous exposure to air pollution.

2. A cluster of mango trees showing the effect of air pollution. The tree at the center is still completely defoliated while those on both sides are recovering.

3. The effects of air pollution on an apparently unaffected chico tree on the left while the santol tree is completely defoliated and on its dying stage. The corn plants in the foreground are wilting and dying.

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1 2

PLATE 1.

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[New names and new combinations are printed in italics.]

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